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<b>(21) International Application Number:</b> PCT/EP98/06977  <b>(22) International Filing Date:</b> 9 October 1998 (09.10.98)  <b>(30) Priority Data:</b> PO 9745 10 October 1997 (10.10.97) AU  <b>(71) Applicant (for all designated States except US):</b> RHONE-POULENC AGRO [FR/FR]; 14/20, rue Pierre Baizet, F-69009 Lyon (FR).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> DOUTRIAUX, Marie-Pascale [FR/FR]; 64, route de Villebon, F-91160 Saulx les Chartreux (FR). BETZNER, Andreas, Stefan [AU/AU]; 40 Dallachy Place, Page, ACT 2614 (AU). FREYSSINET, Georges [FR/FR]; 21, rue de Nervieux, F-69450 Saint Cyr au Mont d'Or (FR). PEREZ, Pascal [FR/FR]; 17, chemin de la Pradelle, Varennes, F-63450 Chanonat (FR).  <b>(74) Agent:</b> GENIN, Patrick; Rhône-Poulenc Agro, DPI, 14/20, rue Pierre Baizet, F-69009 Lyon (FR).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> METHODS FOR OBTAINING PLANT VARIETIES  <b>(57) Abstract</b>  An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.		

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## Methods for Obtaining Plant Varieties

### TECHNICAL FIELD

The present invention relates to nucleotide sequences which encode polypeptides involved in the DNA mismatch repair systems of plants, and to the polypeptides encoded  
5 by those nucleotide sequences. The invention also relates to nucleotide sequences and polypeptide sequences for use in altering the DNA mismatch repair system in plants. The invention also relates to a process for altering the DNA mismatch repair system of a plant cell, to a process for increasing genetic variations in plants and to processes for obtaining plants having a desired characteristic.

### BACKGROUND OF THE INVENTION

Plant breeding essentially relies on and makes use of genetic variation which occurs naturally within and between members of a family, a genus, a species or a subspecies. Another source of genetic variation is the introduction of genes from other organisms which may or may not be related to the host plant.

15 Allelic loci or non-allelic genes which constitute or contribute to desired quantitative (e.g. growth performance, yield, etc.) or qualitative (e.g. deposition, content and composition of seed storage products; pathogen resistance genes; etc.) traits that are absent, incomplete or inefficient in a species or subspecies of interest are typically introduced by the plant breeder from other species or subspecies, or *de novo*. This  
20 introduction is often done by crossing, provided that the species to be crossed are sexually compatible. Other means of introducing genomes, individual chromosomes or genes into plant cells or plants are well known in the art. They include cell fusion, chemically aided transfection (Schocher et al., 1986, Biotechnology 4: 1093) and ballistic (McCabe et al., 1988, Biotechnology 6: 923), microinjection (Neuhaus et al., 1987, TAG 75: 30),  
25 electroporation of protoplasts (Chupeau et al., 1989, Biotechnology 7: 53) or microbial transformation methods such as Agrobacterium mediated transformation (Horsch et al., 1985, Science 227: 1229; Hiei et al., 1996, Biotechnology 14: 745).

However, when a foreign genome, chromosome or gene is introduced into a plant, it will often segregate in subsequent generations from the genome of the recipient plant or  
30 plant cell during mitotic and meiotic cell divisions and, in consequence, become lost from the host plant or plant cell into which it had been introduced. Occasionally, however, the introduced genome, chromosome or gene physically combines entirely or in part with the genome, chromosome or gene of the host plant or plant cell in a process which is called recombination.

35 Recombination involves the exchange of covalent linkages between DNA molecules in regions of identical or similar sequence. It is referred to here as homologous recombination if donor and recipient DNA are identical or nearly identical (at least 99%

base sequence identity), and as homeologous recombination if donor and recipient DNA are not identical but are similar (less than 99% base sequence identity).

The ability of two genomes, chromosomes or genes to recombine is known to depend largely on the evolutionary relation between them and thus on the degree of sequence similarity between the two DNA molecules. Whereas homologous recombination is frequently observed during mitosis and meiosis, homeologous recombination is rarely or never seen.

From a breeder's perspective, the limits within which homologous recombination occurs, therefore, define a genetic barrier between species, varieties or lines, in contrast to homeologous recombination which can break this barrier. Homeologous recombination is thus of great importance for plant breeding. Accordingly there is a need for a process for enhancing the frequency of homeologous recombination in plants. In particular, there is a need for a process of increasing homeologous recombination to significantly shorten the length of breeding programs by reducing the number of crosses required to obtain an otherwise rare recombination event.

At least in *Escherichia coli*, homologous and homeologous recombination are known to share a common pathway that requires among others the proteins RecA, RecB, RecC, RecD and makes use of the SOS induced RuvA and RuvB, respectively. It has been suggested that mating induced recombination follows the Double-Strand Break Repair model (Szostak et al., 1983, Cell 33, 25-35), which is widely used to describe genetic recombination in eukaryotes. Following the alignment of homologous or homeologous DNA double helices the RecA protein mediates an exchange of a single DNA strand from the donor helix to the aligned recipient DNA helix. The incoming strand screens the recipient helix for sequence complementarity, seeking to form a heteroduplex by hydrogen bonding the complementary strand. The displaced homologous or homeologous strand of the recipient helix is guided into the donor helix where it base pairs with its counterpart strand to form a second heteroduplex. The resulting branch point then migrates along the aligned chromosomes thereby elongating and thus stabilising the initial heteroduplexes. Single stranded gaps (if present) are closed by DNA synthesis. The strand cross overs (Holliday junction) are eventually resolved enzymatically to yield the recombination products.

Although in wild type *E. coli* homologous and homeologous recombination are thus mechanistically similar if not identical, homologous recombination in conjugational crosses *E. coli* x *E. coli* occurs five orders of magnitude more frequently than homeologous recombination in conjugational crosses *E. coli* x *S. typhimurium* (Matic et al. 1995; Cell 80, 507-515). The imbalance in favour of homologous recombination was shown to be caused largely by the bacterial MisMatch Repair (MMR) system since its

inactivation increased the frequency of homeologous recombination in *E. coli* up to 1000 fold (Rayssiguier et al. 1989, Nature 342, 396-401).

In *E. coli*, the MMR system (reviewed by Modrich 1991, Annual Rev Genetics 25, 229-253) is composed of only three proteins known as MutS, MutL and MutH. MutS  
5 recognizes and binds to base pair mismatches. MutL then forms a stable complex with mismatch bound MutS. This protein complex now activates the MutH intrinsic single stranded endonuclease which nicks the strand containing the misplaced base and thereby prepares the template for DNA repair enzymes.

During recombination, MMR components inhibit homeologous recombination. In  
10 vitro experiments demonstrated that MutS in complex with MutL binds to mismatches at the recombination branch point and physically blocks RecA mediated strand exchange and heteroduplex formation (Worth et al., 1994; PNAS 91, 3238-3241). Interestingly, the SOS dependent RuvAB mediated branch migration is insensitive to MutS/MutL, explaining the observed slight increase in SOS dependent homeologous recombination.  
15 Homeologous mating even induces the SOS response, thereby taking advantage of RuvAB induction (Matic et al. 1995, Cell 80, 507-515).

The MMR system thus appears to be a genetic guardian over genome stability in *E. coli*. In this role it essentially determines the extent to which genetic isolation, that is, speciation, occurs. The diminished sensitivity of the SOS system to MMR, however,  
20 allows (within limits) for rapid genomic changes at times of stress, providing the means for fast adaptation to altered environmental conditions and thus contributing to intraspecies genetic variation and species evolution.

The important role of MMR in preserving genomic integrity has been established also in certain eukaryotes. In its efficiency, the human MMR, for example, may even  
25 counteract potential gene therapy tools such as triple-helix forming oligonucleotides including RNA-DNA hybrid molecules (Havre et al., 1993, J. Virology 67: 7234-7331; Wang et al., 1995, Mol. Cell. Biol. 15: 1759-1768; Kotani et al., 1996, Mol. Gen. Genetics 250: 626-634; Cole-Strauss et al., 1996, Science 273: 1387-1389). Such oligonucleotides are designed to introduce single base changes into selected DNA target  
30 sequences in order to inactivate for example cancer genes or to restore their normal function. The resulting base mismatches however are recognised by the mismatch repair system which then directs removal of the mismatched base, thereby reducing the efficiency of oligonucleotide induced site-specific mutagenesis.

To date, two families of related genes, homologous to the bacterial *MutS* and *MutL*  
35 genes have been identified or isolated in yeast and mammals (recent reviews by Arnheim and Shibata, 1997, Curr. Opinion Genet. Dev. 7, 364-370; Modrich and Lahue, 1996, Annual Rev. Biochem. 65, 101-133; Umar and Kunkel, 1996, Eur. J. Biochem. 238, 297-307). Biochemical and genetic analysis indicated that eukaryotic MutS homologs (MSH)

and MutL homologs (MLH, PMS), respectively, fulfil similar protein functions as their bacterial counterparts. Their relative abundance, however, could reflect different mismatch specificity and/or specialisation for different tissues or organelles or developmental processes such as mitotic versus meiotic recombination.

5 To date, six different genes homologous to *MutS* have been isolated in yeast (*yMSH*), and their homologs have been found in mouse (*mMSH*) and human (*hMSH*), respectively. Encoded proteins *yMSH2*, *yMSH3* and *yMSH6* appear to be the main *MutS* homologs involved in MMR during mitosis and meiosis in yeast, where the complementary proteins *MSH3* and *MSH6* alternatively associate with *MSH2* to recognise  
10 different mismatch substrates (Masischky et al., 1996, *Genes Dev.* 10, 407-420). Similar protein interactions have been demonstrated for the human homologs *hMSH2*, *hMSH3* and *hMSH6* (Acharya et al., 1996, *PNAS* 93, 13629-13634).

*MutL* homologs (*MLH* and *PMS*), recently reviewed by Modrich and Lahue (1996, *Annual Rev. Biochem.* 65, 101-133) have so far been found in yeast (*yMLH1* and  
15 *yPMS1*), mouse (*mPMS2*) and human (*hMLH1*, *hPMS1* and *hPMS2*). The *hPMS2* is a member of a family of at least 7 genes (Horii et al., 1994, *Biochem. Biophys. Res. Commun.* 204, 1257-1264) and its gene product is most closely related to *yPMS1*. Prolla et al. (1994, *Science* 265, 1091-1093) presented evidence for *yPMS1* and *yMLH1* to physically associate with each other and, together, to interact with the *MutS* homolog  
20 *yMSH2* to form a ternary complex involved in mismatch substrate binding.

However, while medical interest in mismatch repair has prompted extensive research on MMR in bacteria, yeast and mammals, MMR genes have not been isolated from higher plants prior to the present invention and no attempts to adjust the plant MMR to plant breeding needs have been reported.

25

## SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant. In one form of this embodiment, the invention provides an isolated and purified DNA molecule comprising a  
30 polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human. More particularly, the invention provides polynucleotide sequences encoding polypeptides which are homologous to the mismatch repair polypeptides *MSH3* and *MSH6* of *Saccharomyces cerevisiae*. Still more particularly, the invention provides the coding sequences of the genes *AtMSH3* and  
35 *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow, and polynucleotide sequences encoding polypeptides which are homologous to polypeptides encoded by *AtMSH3* and *AtMSH6*.

According to a second embodiment of the invention, there is provided an isolated and purified polypeptide functionally involved in the DNA mismatch repair system of a plant, for example a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human such as a polypeptide encoded by the genes *AtMSH3* or *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow.

According to a third embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is  
10 homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

According to a fourth embodiment of the invention there is provided a chimeric gene comprising a DNA sequence selected from the group consisting of (i) a sequence encoding  
15 a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant: together with at least one regulation element capable of  
20 functioning in a plant cell. Examples of such regulation elements include constitutive, inducible, tissue type specific and cell type specific promoters such as 35S, NOS, PR1a, AoPR1 and DMC1. Typically, a chimeric gene of the fourth embodiment will also include at least one terminator sequence, more typically exactly one terminator sequence.

In the third and fourth embodiments, said interference, by said polynucleotide  
25 sequence, with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair peptide of a yeast or a human typically occurs by hybridisation or by co-suppression.

According to a fifth embodiment of the invention there is provided a plasmid or vector comprising a chimeric gene of the fourth embodiment. A vector of the fifth  
30 embodiment may be, for example, a viral vector or a bacterial vector.

According to a sixth embodiment of the invention, there is provided a plant cell stably transformed, transfected or electroporated with a plasmid or vector of the fifth embodiment.

According to seventh embodiment of the invention, there is provided a plant  
35 comprising a cell of the sixth embodiment.

According to an eighth embodiment of the invention, there is provided a process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising

transforming or transfecting said plant cell with a DNA sequence of the third embodiment or a chimeric gene of the fourth embodiment or a plasmid or vector of the fifth embodiment, and causing said DNA sequence to express said polynucleotide or said polypeptide.

5 According to a ninth embodiment of the invention, there is provided a process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant; permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for  
10 plants in which homeologous recombination has occurred. For example, homeologous recombination may be evidenced by new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait.

According to a tenth embodiment of the invention there is provided a process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair  
15 system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening said plants for a plant having said desired characteristic.

In a preferred form of the ninth and tenth embodiments of the invention, the step of  
20 altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene of the fourth embodiment and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid  
25 plant or cells.

In other embodiments, the invention provides (a) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule of the first embodiment; (b) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO: 18 and (c) an oligonucleotide capable of  
30 hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30; with the proviso that the oligonucleotide of (a), (b) and (c) is other than SEQ ID NO:1 or SEQ ID NO:2.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a diagrammatic representation of the primer sequences used to  
35 isolate *AtMSH3*.

Figure 2 is a plasmid map of clone 52, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH3*.



Figure 3 is a plasmid map of clone 13, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH3*.

Figure 4 is a sequence listing of the coding sequence of *AtMSH3*, together with a deduced sequence of the encoded polypeptide.

5 Figure 5 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH3 protein.

Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*.

10 Figure 7 is a plasmid map of clone 43, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH6*.

Figure 8 is a plasmid map of clone 62, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH6*.

Figure 9 is a sequence listing of the coding sequence of *AtMSH6*, together with a deduced sequence of the encoded polypeptide.

15 Figure 10 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH6 protein.

Figure 11 is a genomic sequence listing of *AtMSH6*.

Figure 12 is a plasmid map of plasmid pPF13.

Figure 13 is a plasmid map of plasmid pPF14.

20 Figure 14 is a plasmid map of plasmid pCW186.

Figure 15 is a plasmid map of plasmid pCW187.

Figure 16 is a plasmid map of plasmid pPF66.

Figure 17 is a plasmid map of plasmid pPF57.

Figure 18 is a diagrammatic representation of an antisense gene construction for use  
25 in homologous meiotic recombination.

Figure 19 is a plasmid map of plasmid p3243.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the inventors' discovery that there exist in higher plants genes which are homologous to MMR genes in *E. coli*, and to MMR genes in  
30 yeasts and humans.

Thus, the inventors have identified genes, herein designated *AtMSH3* and *AtMSH6*, of the plant *Arabidopsis thaliana* which encode the proteins *AtMSH3* and *AtMSH6*. These plant proteins are homologous to *yMSH3* and *yMSH6*, respectively. The present inventors have isolated cDNAs encoding the proteins *AtMSH3* and *AtMSH6* and have  
35 isolated the complete gene encoding *AtMSH6*. Given the teaching herein, other genes (for example *AtMSH2*, and genes of other plants) may be obtained which are involved in DNA mismatch repair in plants, including other genes which encode polypeptides homologous to MMR proteins of yeasts or humans, such as genes which encode

polypeptides homologous to yeast MSH2, MLH1 or PMS2, or to human MLH1, PMS1 or PMS2. For example, given the teaching herein, genes of members of the *Brassicaceae* family or of other unrelated families, for example the *Poaceae*, the *Solanaceae*, the *Asteraceae*, the *Malvaceae*, the *Fabaceae*, the *Linaceae*, the *Canabinaceae*, the *Dauaceae* and the *Cucurbitaceae* family, and which encode polypeptides homologous to MMR proteins of yeasts or humans may be obtained.

Examples of plants whose genes encoding polypeptides homologous to MMR proteins of yeasts or humans may be obtained given the teaching herein include maize, wheat, oats, barley, rice, tomato, potato, tobacco, capsicum, sunflower, lettuce, artichoke, safflower, cotton, okra, beans of many kinds including soybean, peas, melon, squash, cucumber, oilseed rape, broccoli, cauliflower, cabbage, flax, hemp, hops and carrot.

Within the meaning of the present invention, a first polypeptide is defined as homologous to a second polypeptide if the amino acid sequence of the first polypeptide exhibits a similarity of at least 50% on the polypeptide level to the amino acid sequence of the second polypeptide.

A procedure which may be followed to obtain genes *AtMSH3* and *AtMSH6* is described in Example 1. Essentially the same technique may be applied to obtain other mismatch repair genes of *Arabidopsis thaliana*, and essentially the same technique as exemplified herein may be applied to cDNA obtained by reverse transcription of RNA from other plants. Alternatively, given the sequence information disclosed herein, other degenerate oligonucleotide primers, especially oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions (such as the conditions described in Example 1 using primers UPMU and DOMU) to *AtMSH3* and/or *AtMSH6* may be designed and obtained for use in isolating sequences of plant mismatch repair genes which are homologous to *AtMSH3* or *AtMSH6*, from other plants. Similarly, oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions to plant mismatch repair genes of plants other than *Arabidopsis thaliana* also fall within the scope of the present invention and may be utilised to obtain mismatch repair genes of still other plants. Typically, such oligonucleotides are capable of hybridising at 45°C under standard PCR conditions to a DNA molecule which encodes a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or a human. The temperature at which oligonucleotides of the invention hybridise to *AtMSH3* and/or *AtMSH6*, or to plant mismatch repair genes of plants other than *Arabidopsis thaliana*, or to DNA molecules which encode polypeptides which are homologous to a mismatch repair polypeptide of a yeast or a human may be higher than 45°C, for example at least 50°C, or at least 55°C, or at least 60°C or as high as 65°C.

The successful gene isolation disclosed herein demonstrates for the first time the existence of MMR in higher plants and indicates the presence of other plant MMR genes. For example, genes encoding the plant homologs of MSH1, MSH2, MSH4, MSH5, PMS1, PMS2 and MLH1 may be identified given the teaching herein. Such genes, as well as those specifically described herein, separately or in combination, are useful in manipulating the plant MMR for plant breeding purposes. Thus, for example, the plant MMR may be altered by including in a plant cell a polynucleotide sequence as defined herein above with reference to the third embodiment of the invention, and causing the polynucleotide sequence to express either a polynucleotide which disables a plant MMR gene, or a polypeptide which disrupts the plant's MMR system.

The DNA molecule of the third embodiment of the invention includes a polynucleotide sequence (herein referred to as a MMR altering gene) which may for example encode sense, antisense or ribozyme molecules characterised by sufficient base sequence similarity or complementarity to the gene to be altered to permit the antisense or ribozyme molecule to hybridise with the plant MMR gene in vivo or to permit the sense molecule to participate in co-suppression. Alternatively, the MMR altering gene may encode a protein or proteins which interfere with the activity of a plant MMR protein and thus disrupt the plant's MMR system. For example, such encoded proteins may be antibodies or other proteins capable of interfering with MMR protein function, such as by complexing with a protein functionally involved in plant MMR thereby disrupting the MMR of the plant. An example of such a protein is the MSH3 protein of *Arabidopsis thaliana* described herein or a protein of another plant which is homologous to the MSH3 protein of *A. thaliana*. For instance, overexpression of MSH3 in a plant cell causes MSH2 present in the cell to be substantially completely complexed, disrupting the mismatch repair mechanism or mechanisms in the cell which are functionally dependent on the presence of a complex of MSH2 with MSH6. Similarly, mismatch repair mechanisms which depend on the presence of a complex of MSH2 and MSH3 may be disrupted by the overexpression of MSH6.

A chimeric gene of the fourth embodiment, incorporating a MMR altering gene, may be prepared by methods which are known in the art. Similarly, the MMR altering gene may be introduced into a plant cell, regenerating tissue or whole plant by techniques known in the art as being suitable for plant transformation, or by crossing. Known transformation techniques include *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters. Suitable promoters may direct constitutive expression, such as the 35S or the NOS promoter. Usually, however, the promoter will direct either inducible or tissue specific (e.g. callus; embryonic tissue; etc.), cell type specific (e.g. protoplasts; meiocytes; etc.) or developmental (e.g. embryo) expression of the altering gene or genes, in order for the

MMR system to function in tissue types or cell types, or at developmental stages of the plant, in which it is not desirable for the MMR system to be altered. Using such promoters, therefore, the activity of a MMR altering gene may be limited to a specific stage during plant development or it may be altered by controlling conditions external to the plant, and the deleterious effects of a permanently disabled or altered DNA mismatch repair system in a plant may be avoided. Examples of suitable promoters which are not constitutive are known in the art and include inducible promoters such as *PR1a* (reviewed by Gatz, 1997, Annual Rev. Plant Phys. Plant Mol. Biol. 48: 89), tissue specific promoters such as *AtPRI* (Sabahattin et al., 1993, Biotechnology 11: 218), and cell-type specific promoters such as *DMC1*.

A chimeric gene in accordance with the invention may further be physically linked to one or more selection markers such as genes which confer phenotypic traits such as herbicide resistance, antibiotic resistance or disease resistance, or which confer some other recognisable trait such as male sterility, male fertility, grain size, colour, growth rate, flowering time, ripening time, etc.

The process of the tenth embodiment of the invention provides, for example, a process for generating intraspecies genetic variation by altering the mismatch repair system in a plant cell, in regenerating plant tissue or in a whole plant. The plant cell, regenerating tissue or whole plant includes and expresses one or more MMR altering genes which are capable of altering mismatch repair in the plant cell, regenerating tissue or whole plant. Alteration of MMR may be achieved, for example, by inactivating the genes encoding plant MSH3 and/or plant MSH6. It is preferred to inactivate the plant MSH3 and MSH6 encoding genes at the same time and in the same plant cell, regenerating tissue or whole plant. Typically in this preferred form of the invention inactivation of either plant MSH3 or MSH6 alone is insufficient to substantially alter the plant's mismatch repair system and only when both MSH3 and MSH6 are inactivated simultaneously is the plant's mismatch repair system sufficiently altered to prevent the MMR system from recognising base pair mismatches, base insertions or deletions as a result of DNA replication errors, DNA damage, or oligonucleotide induced site-specific mutagenesis. However, in some applications of the invention, inactivation of only one gene may also be used to cause genomic instability or increase the efficiency of site-specific mutagenesis.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the genome of the plant cell, regenerating tissue or whole plant in order to restore mismatch repair in the plant cell, regenerating tissue or whole plant. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools, such as ribozymes, or may be removed from the genome using gene elimination systems known in the art, such as *CRE/LOX*. It is preferred to render two genes, whose gene products combine to incapacitate MMR, ineffective by separating

the altering genes through segregation. Therefore, in a preferred embodiment of the invention a first plant cell or plant is generated in which only plant *MSH3* is incapacitated, and a second plant cell or plant is generated in which only plant *MSH6* is incapacitated. The combination of both genomes, for example by crossing, then produces significant  
5 MMR deficiency in those cells or plants which have inherited both altering genes. If the altering genes are expressed from unlinked loci, gene segregation restores MMR activity in the progeny of the cells or plants.

In a process of the ninth embodiment of this invention, homeologous recombination is enhanced between different genomes, chromosomes or genes in plant cells or plants by  
10 altering MMR in said plant cells or plants. Such genomes, chromosomes or genes are characterised in that they originate from different plant families, genera, species, subspecies, plant varieties or lines. Hybrid plant cells or hybrid plants may be produced by crossing, by cell fusion or by other techniques known in the art. These plant cells or plants are further characterised by expressing one or more genes that are capable of  
15 altering mismatch repair in the plant cell or plants.

In the process of the ninth embodiment, the homeologous recombination is typically for the purpose of introducing a desired characteristic in the hybrid plant. In this typical application of the process of the ninth embodiment, and in the process of the tenth embodiment the desired characteristic may be any characteristic which is of value to the  
20 plant breeder. Examples of such characteristics are well known in the art and include altered composition or quality of leaf or seed derived storage products (e.g. oil, starch, protein), altered composition or quality of cell walls (e.g. decrease in lignin content), altered growth rate, prolonged flowering, increased plant yield or grain yield, altered plant morphology, resistance to pathogens, tolerance to or improved performance under  
25 environmental stresses of various kinds, etc.

In a preferred form of the tenth embodiment, an MMR altering gene is co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant to produce a hybrid plant cell or hybrid plant in which homeologous recombination can occur.  
30 Suitably, the MMR proficient plant cell or MMR proficient plant may also include an MMR altering gene. For example a gene capable of inactivating plant *MSH3* may be co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant in which *MSH6* is inactivated. A resultant hybrid plant in which homeologous recombination occurs will  
35 include both the *MSH3* and *MSH6* altering genes and its MMR system will therefore be inactivated.

In this form of the invention, if hybrid plants are to be produced by crossing, the MMR altering gene preferably originates from the male parent, thus ensuring that the

MMR altering gene is always introduced and is not present in the recipient cell. That is, the MMR of the recipient cell, prior to introduction of the MMR altering gene, is typically proficient. Alternatively, if an MMR altering gene is present in a recipient cell it may be ineffective or inefficient on its own, or it may be linked to an inducible or tissue specific or cell type specific promoter which only renders the MMR altering gene active under limited conditions.

Thus, in a preferred form of the process of the ninth embodiment, the MMR system of the hybrid plant is initially unaltered. In this form of the process, the step of altering the mismatch repair system may comprise introducing into the hybrid plant, or cells thereof, a MMR altering gene, such as by *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters, as described above. Preferably, the promoter is transcriptionally active in mitotically and meiotically active tissue and/or cells to ensure MMR alteration after chromosome pairing at mitosis and meiosis, respectively. The preferred timing for MMR alteration is at meiosis, because recombinant genomes, chromosomes or genes are directly transmitted to the progeny. A suitable meiocyte specific promoter is for example the *DMC1* promoter from *Arabidopsis thaliana* ssp. *Ler.* (Klimyuk and Jones, 1997, Plant J. 11, 1-14). However, mitotic homeologous recombination is also a desirable outcome as somatic recombination events can be transmitted to offspring due to the totipotency of plant cells and the lack of predetermined germ cells in plants.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the hybrid plant or hybrid plant cells, in order to restore mismatch repair in the hybrid plant or hybrid plant cells. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools as described herein above.

## EXAMPLES

### Example 1. Cloning of the *AtMSH3* and *AtMSH6* coding sequences

#### Isolation of partial *AtMSH3* and *AtMSH6* consensus sequences

Degenerate oligonucleotides UPMU (SEQ ID NO:1) and DOMU (SEQ ID NO:2)

UPMU CTGGATCCACIGGICCAA(C/T)ATG

DOMU CTGGATCC(A/G)TA(A/G)TGIGTI(A/G)C(A/G)AA

were used to isolate *AtMSH3* and *AtMSH6* sequences by PCR amplification.

Primers UPMU and DOMU correspond to conserved amino acid sequences of the proteins MutS (*E. coli* and *S. typhimurium*), HexA (*S. pneumoniae*), Rep1 (mouse) and Dc1 (human). The conserved regions to which they are targeted are TGPNM for UPMU (amino acid positions 852-856 for *AtMSH6* and 816-820 for *AtMSH3*) FATHY or FVTHY

for DOMU (amino acid positions 964-968 for AtMSH6 and 928-932 for AtMSH3, respectively.) These primers have been used to isolate MSH2 and MSH1 from yeast (Reenan and Kolodner, Genetics 132: 963-973 (1992)) and MSH2 from *Xenopus* and mouse (Varlet et al., Nuc. Acids Res. 22:5723-5728 (1994)).

- 5 Template single strand cDNA was produced by reverse transcription of 2 µg total RNA from a cell suspension culture of *Arabidopsis thaliana* ecotype Columbia (Axelos et al. 1989, Mol. Gen. Genetics 219: 106-112). The PCR reaction was performed under the following conditions in a final volume of 100µl: 0.2mM dNTP, 1µM each primer, 1XPCR buffer, 1u *Taq* DNA polymerase (Appligene) in the presence of template cDNA. PCR
- 10 parameters were 5 minutes at 94°C, followed by 30 cycles of 40 seconds at 95°C, 90 seconds at 45°C, 1 minute at 72°C. The amplification products were cloned into pGEM-T vector (Promega) and sequenced. Two different clones were isolated, S5 (350bp) was homologous to *MSH3*, S8 (327bp) was homologous to *MSH6*. Complete cDNA sequences were then isolated according to the Marathon cDNA amplification kit procedure (Clontech).
- 15 In summary, this procedure involves producing double stranded cDNA by reverse transcription of 2µg polyA<sup>+</sup> RNA from the cell suspension culture of *Arabidopsis*. Adaptors are ligated on each side of the cDNA. The ligated cDNA is used as a template for 5' and 3' RACE PCR reactions in the presence of primers that are specific for the adaptor on one side (AP1 and AP2), and specific for the targeted gene on the other side. A 5' and a 3'
- 20 fragment that overlap are thus produced for each gene. The complete gene coding sequence can be reconstituted taking advantage of a unique restriction site, if available, in the overlapping region. Specific details of this procedure as it was used to isolate *AtMSH3* and *AtMSH6* coding regions, are as follows.

#### Isolation of *AtMSH3* complete coding sequence

- 25 From the sequence of clone S5, primer 636 (SEQ ID NO:3) was designed:

636 TGCTAGTGCCTCTTGCAAGCTCAT.

Primer AP1 (SEQ ID NO:4) is complementary to a portion of an adaptor sequence which had been ligated to the 5' and 3' ends of *Arabidopsis* cDNA:

AP1 CCATCCTAATACGACTCACTATAGGGC.

- 30 PCR performed on the ligated cDNA with primers 636 and AP1 for the 5' RACE PCR was followed by a second round of amplification with the nested primers AP2 (SEQ ID NO:5) and S525 (SEQ ID NO:6)

AP2 ACTCACTATAGGGCTCGAGCGGC

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA

- 35 (the latter was also designed to correspond to a part of the sequence of clone S5) and produced a 2720bp DNA fragment. Figure 1 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH3*. Another primer (S51, SEQ ID NO:7)

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG

was designed closer to the 5' border and permitted the determination of 99bp upstream to the ATG initiation codon. For the 3' RACE PCR, a first PCR reaction was performed with primers AP1 and 635 (SEQ ID NO:8).

635 GCACGTGCTTGATGGTGTTCAC

5 followed by a second round of amplification, using the nested primers AP2 and S523 (SEQ ID NO:9)

S523 TCAGACAGTATCCAGCATGGCAGAAGTA

which produced a DNA fragment of 890bp. Both DNA fragments were subcloned into pGEM-T and sequenced. Since PCR amplification using the Expand Long Template PCR  
10 System (Boehringer-Mannheim) produced errors in the sequence, new oligonucleotides were designed to isolate those sequences again by PCR, but with the high fidelity DNA polymerase *Pfu*. PCR with primers 1S5 (SEQ ID NO:10) and S53 (SEQ ID NO:11)

1S5 ATCCCGGGATGGGCAAGCAAAGCAGCAGACGA

S53 GACAAAGAGCGAAATGAGGCCCTTGG

15 amplified the 1244bp fragment clone 52 (SEQ ID NO:12, cloned into pUC18/*Sma*I). PCR with primers S52 (SEQ ID NO:13) and 2S5 (SEQ ID NO:14)

2S5 ATCCCGGGTCAAATGAACAAGTTGGTTTATGTC

S52 GCCACATCTGACTGTTCAAGCCCTCGC

amplified the 2104bp clone 13 (SEQ ID NO:15, cloned into pUC18/*Sma*I). The complete  
20 coding sequence of the *AtMSH3* gene was reconstructed in pUC18 by ligating the 5' half of *AtMSH3* (clone 52) to the 3' half of *AtMSH3* (clone 13) after digesting with *Bam*HI which has a unique cleavage site in the overlapping region of both clones. This manipulation yielded plasmid pPF26. The *Sma*I fragment from pPF26 contains the complete *AtMSH3* coding sequence. The remaining primers referred to in Figure 1 are as  
25 follows:

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG (SEQ ID NO:16)

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA (SEQ ID NO:17)

Figures 2 and 3 provide plasmid maps of clones 52 and 13 respectively, showing restriction enzyme cleavage sites. The complete *AtMSH3* coding sequence (SEQ ID NO:18)  
30 is 3246bp long and is shown in Figure 4 together with the deduced sequence (SEQ ID NO:19) of the encoded polypeptide. *AtMSH3* is clearly homologous to the yeast and mouse *MSH3* genes. A sequence alignment of polypeptides encoded by *AtMSH3* and that encoded by *Saccharomyces cerevisiae MSH3* is set out in Figure 5.

#### Isolation of the *AtMSH6* complete coding sequence and genomic sequences

35 The same procedure allowed isolation of the *AtMSH6* cDNA. Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*. For the 5' RACE PCR, primers 638 (SEQ ID NO:20) and AP1 (SEQ ID NO:4)

638 TCTCTACCAGGTGACGAAAACCG

allowed the amplification of a 2889 DNA fragment. Primer S81 (SEQ ID NO:21)



S81 CGTCGCCTTTAGCATCCCCTTCCTTCAC

helped define the 142bp upstream to the ATG initiation codon. On the 3' side, RACE PCR was initially performed with primers S823 (SEQ ID NO:22) and AP1 (SEQ ID NO:4),

S823 GCTTGGCGCATCTAATAGAATCATGACAGG

5 and then with the nested primers 637 (SEQ ID NO:23) and AP2 (SEQ ID NO:5).

637 GACAGCGTCAGTTCTTCAGAATGC

to produce a 774bp DNA fragment. As for *AtMSH3*, those fragments were cloned and sequenced. Re-isolation of the DNA sequence using the high fidelity *Pfu* polymerase and newly designed primers 1S8 (SEQ ID NO:24) and S83 (SEQ ID NO:25) (for the 5' side) led  
10 to a 2182 bp DNA fragment identified as clone 43 (SEQ ID NO:26, cloned in pUC18/SmaI), and a 1379bp clone identified as clone 62 (SEQ ID NO:27, also cloned in pUC18/SmaI).

1S8 ATCCCGGGATGCAGCGCCAGAGATCGATTTTGT

2S8 ATCCCGGGTTATTGGAACACAGTAAGAGGATT (SEQ ID

15 NO:28)

S82 GCGTTCGATCATCAGCCTCTGTGTTGC (SEQ ID NO:29)

S83 CGCTATCTATGGCTGCTTCGAATTGAG

Figures 7 and 8 provide plasmid maps of clones 43 and 62 respectively, showing restriction enzyme cleavage sites. Clones 43 and 62 were digested by the *XmnI* restriction enzyme for  
20 which a unique site is present in their overlapping region and then ligated. The complete *AtMSH6* coding sequence (SEQ ID NO:30) is 3330bp long and is shown in Figure 9 together with the deduced sequence (SEQ ID NO:31) of the encoded polypeptide. *AtMSH6* is clearly homologous to the yeast and mouse *MSH6* genes. A sequence alignment of polypeptides encoded by *AtMSH6* and that encoded by *Saccharomyces cerevisiae MSH6* is  
25 set out in Figure 10.

An *AtMSH6* genomic sequence was also isolated from a genomic DNA library constituted after partial *Sau3AI* digestion of DNA from the *Arabidopsis* cell suspension. 8062bp were sequenced that covered the *AtMSH6* gene and show colinearity with the cDNA. 16 introns are found scattered along the gene. The complete genomic sequence  
30 (SEQ ID NO:98) is shown in Figure 11.

## Example 2. A measure of somatic variation in MMR deficient plants

### Constructs

Constructs with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* under the control of a single 35S promoter have been inserted into the binary vector  
35 pPZP121 (Hajdukiewicz et al., Plant Mol. Biol. 23, 793-799) between the right and left borders of the T-DNA. The pPZP121 plasmid confers chloramphenicol resistance to *Escherichia coli* or *Agrobacterium tumefaciens* bacteria. The *aacCI* gene is carried by the T-DNA and allows selection of transformed plant cells on gentamycin (Hajdukiewicz et al., Plant Mol. Biol. 25, 989-994). For the purpose of expressing antisense constructs, a 35S

promoter/terminator cassette with a polylinker was introduced into pPZP121. The 3' ends of the considered genes have been chosen since this region seems more efficient for antisense inhibition. For *AtMSH3* this corresponds to clone 13 (2104bp). for *AtMSH6* this corresponds to clone 62 (1379bp). Clone 13 comprises 2104bp of the 3' region that were cut  
5 off the pUC18 vector by SalI/SstI restriction, blunted with T4 DNA polymerase and ligated into the T4 DNA polymerase blunted *Bam*HI site of pPZP121/35S, creating clone pPF13. The same procedure was followed for the 3' region of *AtMSH6* clone 62 (1379bp) thus creating plasmid pPF14. For the double constructs, the 3' region (from clone 62) of *AtMSH6* was introduced ahead of the *AtMSH3* region into pPF13 creating pCW186 and  
10 reciprocally, the 3' region of *AtMSH3* (from clone 13) was introduced ahead of *AtMSH6* into pPF14, creating pCW187.

These constructs were introduced into the Arabidopsis cells (as described below) of wildtype Columbia and of the Columbia tester line.

An alternative strategy to antisense inhibition of *AtMSH6* comes from experiments of  
15 Marra et al. (1998, Proc. Natl. Acad. Sci USA 95, 8568-8573) who show that overexpression of functional *MSH3* results in depletion of MSH6 protein in human cells. This depletion may generate a mismatch repair mutant phenotype.

For the purpose of overexpressing functional *AtMSH3* protein in plant cells, the complete *MSH3* coding region was excised from pPF26 (example 1) by digestion with  
20 *Sma*I, and was inserted into the *Sma*I site of pCW164. The resulting construct was named pPF66. It contains a complete *AtMSH3* gene under the control of the 35S promoter inside the left (LB) and right (RB) border of the T-DNA. This T-DNA also contains the *hpt2* gene for gentamycin selection. Plasmid pPF66 was introduced into Arabidopsis cells as described below. One cell clone was selected which clearly overexpressed the *AtMSH3*  
25 gene as shown by Northern analysis. Figures 12-16 provide plasmid maps of plasmids pPF13, pPF14, pCW186, pCW187 and pPF66, respectively.

#### Construction of tester construct

For the purpose of Forward Mutagenesis Assays, a tester construct was built containing the coding regions for *nptII*, *codA*, *uidA*. All three genes are driven by the 35S  
30 promoter and are terminated by the 35S terminator. This construct was obtained by introducing an EcoRI fragment encoding the *codA* cassette (2.5kb) and a *Hind*III fragment encoding the *uidA* (*GUS*) cassette (2.4kb) into the pPZP111 vector (Hajdukiewicz et al., 1994, Plant Mol Biol 23: 793-799) which already contained the *nptII* expression cassette. This new plasmid was named pPF57. *NptII* is used to select for transformed plant cells.  
35 *GUS* is used to analyse the degree of gene silencing in the construct (i.e. to identify cell lines in which the transgenes are expressed), and *codA* is used as a marker for forward mutagenesis (described below).

The plasmid map of pPF57 is provided in Figure 17.

#### Plant cell transformation

The constructs are introduced into *Agrobacterium* by electroporation. Plant cells are then transformed by co-cultivation. A suspension culture of *Arabidopsis thaliana* cells that has been established by Axelos et al. (1992, Plant Physiol. Biochem. 30, 1-6) may be used. One day old freshly subcultured cells are diluted five times into AT medium (Gamborg B5 medium, 30g/l sucrose, 200µg/l NAA). 10µl of saturated *Agrobacterium* containing the transforming T-DNA constructs are added to 10ml diluted cells in a 100ml erlenmeyer. The co-cultivation is agitated slowly (80rpm) for 2 days. The cells are then washed 3 times into AT medium and finally resuspended in the same initial volume (10ml). The culture is agitated for 3 days to allow expression before plating on selection plates (AT/BactoAgar 8g/l+gentamycin 50µg/ml). Transformed individual calli are isolated 3 weeks later.

#### Tester Strain

The tester construct on plasmid pPF57 was introduced into *Arabidopsis* cells of wildtype Columbia using the transformation protocol described above. Among 10 candidate transformants, one cell clone was shown (by Southern analysis) to have a unique T-DNA insertion. All three genes were shown to be functional in this cell line as indicated by resistance to kanamycin, blue staining in the presence of X-Glu (*GUS*), and sensitivity to 5-fluoro-cytosine (*codA*).

MMR altering genes (described above) were then introduced individually into the tester line and transformed cells are used for analysis of both Microsatellite Instability and Forward Mutagenesis.

#### Microsatellite analysis

Microsatellites have been described in *Arabidopsis* (Bell and Ecker, 1994, Genomics 19, 137-144). The present Example is based on a study of instability of microsatellites that are polymorphic amongst different ecotypes. DNA is extracted from the transformed calli. Specific primers have been defined that are used to amplify the microsatellite sequence. One of the two primers is previously P<sup>32</sup> labelled by T4 kinase. In case of a polymorphic variation, new PCR products appear that do not follow the expected pattern of migration on a polyacrylamide gel. This is a commonly observed feature for MMR deficient cells in yeast or mammalian cells.

In particular, the present Example describes a study on microsatellites ca72 (CA<sub>18</sub>), nga172 (GA<sub>29</sub>), and ATHGENEA(A<sub>39</sub>), chosen because they belong to the types predominantly affected in human mismatch repair deficient tumors. The size of these microsatellites is not conserved from one *Arabidopsis* ecotype to the other.

*Arabidopsis* cells which are transformed with an MMR altering gene (above) and control cells not expressing the MMR altering gene are allowed to form calli. DNA is

rapidly extracted from the calli and is analysed for microsatellite instability as described in detail by Bell and Ecker 1994. Genomics 19, 137-144. In summary, the relevant microsatellite is amplified by PCR using P32 labelled primers. The PCR products are separated on a DNA sequencing gel for size determination. Size differences between  
 5 microsatellites from transformed and control cells not expressing the MMR altering gene in question indicate microsatellite instability as a result of MMR alteration.

The sequences of primers used for PCR amplification of microsatellites ca72 and nga172 are included in Table 1. PCR amplification of microsatellite ATHGENEA made use of a forward primer containing the sequence

10           ACCATGCATAGCTTAAACTTCTTG (SEQ ID NO:32)

and of a reverse primer containing the sequence

          ACATAACCACAAATAGGGGTGC (SEQ ID NO:33).

The amplification for microsatellite ca72 revealed in Columbia control cells (with respect to the MMR altering gene) a 248 bp long PCR fragment instead of the published  
 15 length of 124 bp. DNA sequencing verified this fragment as a CA<sub>18</sub> microsatellite.

#### Forward mutagenesis assay

Tester cells transformed with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* are analysed for the stability of the *codA* gene. The functional *codA* gene confers to sensitivity to 5-fluoro-cytosine (5FC), whereas a gene inactivating mutation in  
 20 *codA* will confer resistance to 5FC. The frequency of resistant cells is therefore a good indicator of somatic variation as a direct result of MMR alteration. Variants resistant to 5FC are first analysed for GUS activity. If GUS is inactive, 5FC resistance is assumed to be due to gene silencing (all three genes are under the 35S promoter). If GUS is active, 5FC resistance is assumed to be due to forward mutations that have inactivated *codA*. PCR is  
 25 then performed on the putative *codA* mutant genes which is then sequenced to confirm the presence of forward mutations in *codA*.

Besides *codA*, other marker genes may also be used for the Forward Mutagenesis Assay such as the *ALS* gene (conferring sensitivity to valine or to sulfonylurea; Hervieu and Vaucheret, 1996, Mol. Gen. Genet. 251 220-224; Mazur et al. 1987, Plant Physiol. 85 1110-  
 30 1117).

### **Example 3. Homeologous meiotic recombination in *Arabidopsis thaliana***

#### **A. Construction of a meiocyte specific gene expression cassette comprising the *DMC1* promoter and the *NOS* terminator**

(i) The *DMC1* promoter may be used as published by Klimyuk and Jones, 1997,  
 35 Plant J. 11.1-14). To obtain a more convenient alternative for gene cloning, a 3.3 Kb

long subfragment of the *DMC1* promoter was obtained by PCR from genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "*Ler*").

The PCR was done in three rounds:

Round One: A 3.7 Kb long product was obtained using the forward primer  
5 DMCIN-A comprising the sequence

GAAGCGATATTGTTCTGTG (SEQ ID NO:34)

and the reverse primer DMCIN-B comprising the sequence

AGATTGCGAGAACATTCC (SEQ ID NO:35).

The weak amplification product was then used as template for round two and three.

10 Round Two: A 3.1 Kb long product comprising the promoter and the 5' untranslated leader was obtained using forward primer DMCIN-1, which contained the sequence

acgctcgacTCAGCTATGAGATTACTCGTG (SEQ ID NO:36)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment, and reverse  
15 primer DMCIN-2 which contained the sequence

gctctagaTTTCTCGCTCTAAGACTCTCT (SEQ ID NO:37)

and introduced a *XbaI* site at the 3' end of the PCR fragment.

Round Three: A 0.2 Kb long product comprising the first exon/intron of the *DMC1* promoter was obtained using forward primer DMCIN-3, which contained the sequence

20 gctctagaGCTTCTCTTAAGTAAGTGATTGAT (SEQ ID NO:38)

and introduced a *XbaI* site at the 5' end of the PCR fragment, and reverse primer DMCIN-4, containing the sequence

tccccgggctcgagagatctccatggTTTCTTCAGCTCTATGAATCC (SEQ ID NO:39)

and introduced at the 3' end of the PCR product restriction sites for *NcoI*, *BglII*, *XhoI* and  
25 *SmaI*.

The products obtained in round Two and Three were digested with *XbaI* and subsequently ligated to reconstitute a 3.3 Kb long *DMC1* promoter from which the first two in-frame ATG start codons were replaced with a unique restriction site for *XbaI*. This promoter can be cloned between the restriction sites for *SalI* and *SmaI* of p3264,  
30 which contains the *SacI-EcoRI* NOS terminator in pBIN19, to yield the entire expression cassette in pBIN19. This cassette contains the following cloning sites: *NcoI*, *BglII*, *XhoI*, *SmaI* and (already present on p3264) *KpnI* and *SacI*.

(ii) Another strategy yielded the following convenient *DMC1* promoter. A 1.8 kb DNA fragment comprising the 3' terminal part of the meiocyte specific *DMC1* promoter  
35 was isolated by PCR from purified genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "*Ler*"). The forward PCR primer (DMC1a) contained the sequence

acgctcgacGAATTCGCAAGTGGGG (SEQ ID NO:40)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment. The reverse PCR primer (DMC1b) contained the sequence

tccatggagatctcccggtacCGATTGCTTCGAGGG (SEQ ID NO:41)

introducing a polylinker region at the 3' end of the promoter fragment. The PCR reaction was carried out with VENT DNA Polymerase (NEB) over 25 cycles using the following cycling protocol: 1 minute at 94°C, 1 minute at 54°C, 2 minutes at 72°C.

5 The PCR reaction yielded a blunt ended DNA fragment which was digested with restriction enzyme *SaII* and was cloned into the cleavage sites of restriction enzymes *SaII* and *SmaI* in plasmid p2030, a pUC118 derivative containing the *SacI-EcoRI* NOS terminator fragment of pBIN121. The cloning yielded plasmid p2031, containing the *DMC1* promoter-polylinker-NOS terminator expression cassette depicted in Figure 18.

10 B. Construction of an *MSH3* antisense gene under the control of the *DMC1* promoter

A 2.1 kb DNA fragment encoding the carboxyterminal part of AtMSH3 was removed from the polylinker of clone 13 described in Example 1 after (i) digestion with *KpnI*, (ii) blunting of the DNA ends generated by *KpnI* and (iii) digestion with *BamHI*. The isolated fragment was then cloned in antisense orientation downstream of the *DMC1* promoter in plasmid p2031, which had been digested with restriction enzymes *SmaI* and *BgIII*. This cloning yielded plasmid p2033 (Figure 18).

After digestion of p2033 with *EcoRI*, a 4.1 kb DNA fragment was recovered comprising the *DMC1* promoter, the partial *MSH3* cDNA in antisense orientation with respect to the promoter and the *NOS* terminator. This fragment was cloned into the *EcoRI* restriction site of plant transformation vector pNOS-Hyg-SCV to yield plasmid p3242 (Figure 18).

C. Construction of a combined *MSH6/MSH3* antisense gene under the control of a single *DMC1* promoter

A 3.1 kb fragment, encoding in antisense orientation the partial AtMSH6 and AtMSH3 sequences and the 35S terminator, was isolated from pCW186 by digestion with *KpnI*. The fragment was treated with *Klenow* enzyme to blunt both ends. It was then cloned into the *SmaI* site of plasmid p3243 (a pNOS-Hyg-SCV derivative, illustrated in Figure 19), which had been opened to delete the region between the *SmaI* sites. Clones containing the fragment in the antisense orientation with respect to the *DMC1* promoter (described in A(ii) above) were identified by diagnostic digestion with *BamHI*. The selected construct was named p3261.

Another practical way of cloning the double antisense gene is as follows. A 1 kb DNA fragment encoding the carboxyterminal part of AtMSH6 is isolated from clone 62 described in Example 1 after digestion of clone 62 plasmid DNA with *BamHI*, which cleaves in the 5' polylinker region flanking the partial cDNA, and with *EcoRI*, which cleaves within the cDNA. The isolated fragment is treated with *Klenow* enzyme to blunt both its ends and is cloned into the recipient plasmid p2033 or p3242. For the purpose of

cloning, the recipient plasmid may be cleaved with either *Ava*I or *Nco*I and can be blunted with *Klenow* enzyme to produce blunt acceptor ends for fragment cloning. This cloning yields two opposite orientations of cloned fragment DNA with respect to the *DMC1* promoter. These can be identified by diagnostic digestion with restriction enzymes *Sca*I or *Xmn*I in conjunction with *Sac*I. The selected construct contains the *DMC1* promoter, the combined partial cDNAs for *AtMSH3* and *AtMSH6* (both cloned in antisense orientation with respect to the *DMC1* promoter) and the *NOS* terminator. If the recipient plasmid is p2033, the combined antisense gene under control the single *DMC1* promoter is recovered from the vector after *Eco*RI digestion and is cloned into the *Eco*RI restriction site of pNOS-Hyg-SCV.

D. Construction of a full-length *MSH3* sense gene under control of the *DMC1* promoter for overexpression of functional *MSH3* protein

Overexpression of *MSH3* protein was shown in human cells (Marra et al., 1998, Proc. Natl. Acad. Sci. USA 95, 8568-8573) to complex all available *MSH2* protein. This leaves *MSH6* protein without its partner, leading to the degradation of *MSH6* protein and eventually to a mismatch repair phenotype.

This phenomenon is exploited to increase homeologous meiotic recombination in *Arabidopsis* as an alternative to antisense inhibition of *AtMSH6*. For this purpose the full-length cDNA encoding *AtMSH3* is isolated from plasmid pPF66 by digestion with *Sma*I and is cloned into the *Sma*I site of the *DMC1* expression cassettes described in A(i).

E. Selection of Recombination markers on homeologous chromosomes of *Arabidopsis thaliana* subspecies *Landsberg erecta* (Ler), *Columbia* (Col) and *C24*, respectively

E(i). Visual recombination markers in *Arabidopsis th.* subspecies *C24*:

*Agrobacterium* mediated transformation with a T-DNA containing a 35S-*GUS* gene, inactivated by insertion of a 35S-*Ac* transposable element (Finnegan et al., 1993, Plant Mol. Biol. 22, 625-633), had yielded a *C24* line in which the T-DNA construct was integrated into chromosome 2. Genetic and molecular analysis of this line shows that the *Ac* transposon had excised from its T-DNA locus thereby restoring *GUS* activity, but had re-inserted into the chromosome at a distance of 16.4 cM, where it stayed fixed (due to disablement of *Ac*) within the *chlorina* gene. Insertional inactivation of the *chlorina* gene caused a bleached phenotype in those plants that were homozygous for this mutation. Because of the two linked phenotypic markers, *chlorina3A:Ac* and *GUS*, this *C24* line was used in crosses to wildtype Ler for analysis of meiotic homeologous recombination on chromosome 2 in conjunction with molecular recombination markers.

E(ii). Visual recombination markers in *Arabidopsis th.* *Ler*:

The Ler line NW1 (obtained from NASC, Nottingham, UK) contains one recessive visual marker per chromosome. i.e. *an-1* on Chr.1, *py-1* on Chr.2, *gll-1* on Chr.3, *cer2-1*

on Chr.4, and *msl-1* on Chr.5. This line is used in crosses to wildtype C24 which expresses an MMR altering gene for analysis of meiotic homeologous recombination on chromosomes 1-5 in conjunction with molecular recombination markers listed in Table 1.

Other *Ler* lines from NASC have several visual markers in close proximity to each other on the same chromosome. When these lines are used for hybrid production, analysis of homeologous meiotic recombination may make use entirely of visual recombination markers. Particularly suitable for crossing to C24 wildtype that is expressing a MMR altering gene are the following *Ler* lines:

NW22: relative markers are *dis1* - (4 cM) - *ga4* - (11 cM) - *th1* on chromosome 1.

10 NW10: relevant markers are *tz-201* - (5 cM) - *cer3* on chromosome 5.

NW134, relevant markers are *ttg* - (4 cM) - *ga3* on chromosome 5.

NW24 (*abi3-1*) and NW64 (*gll-1*). When present in the same plant on chromosome 3, *abi3-1* and *gll-1* are 13 cM apart. Since this marker combination is not available from NASC, we have combined these markers by crossing line NW24 to line NW64. The F1 offspring were allowed to self-fertilise and to produce F2 seeds. F2 Plants which carry both markers as homozygous traits on the same chromosome can be identified firstly, by germinating F2 seeds on germination medium containing selective concentrations of abscisic acid, and subsequently, by identifying among the abscisic acid resistant plants those individuals which show the glabra phenotype.

#### 20 E(iii) Molecular recombination markers in *Col*, *Ler* and C24:

The genome of *Arabidopsis thaliana* is interspersed with unique base sequences arranged as simple tandem repeats. Allelic repeats can vary in length between different *Arabidopsis* subspecies and when amplified by PCR yield diagnostic DNA products of different length named Simple Sequence Length Polymorphisms (SSLPs). Many SSLPs have been genetically mapped and have been assigned to unique chromosome locations on the recombinant inbred map (Bell and Ecker, 1994, Genomics 19, 137-144; Lister and Deans lines, Weeds World 4i, May 1997).

In Table 1 are listed 28 mapped and established SSLPs between *Ler* and *Col*. A number of PCR primer pairs are described herein (SEQ ID NO:42 to SEQ ID NO:97) which also yielded SSLPs between C24 and *Ler* (19 SSLPs) or between C24 and *Col* (25 SSLPs), respectively. Polymorphic SSLPs can be used as molecular markers in the analysis of homeologous recombination between genomes from these subspecies.

The PCR reactions (25 µL) were carried out over 35 cycles (15 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C), with 0.25 U Taq DNA polymerase and 0.6 µg genomic DNA in reaction buffer containing 2 mM MgCl<sub>2</sub>. PCR products were separated by agarose gel electrophoresis (4% ultra high resolution agarose) and visualised by ethidiumbromide staining. The results from the PCR experiments are summarised in



Table 1, which also shows the sequence of PCR primers, primer annealing temperature (Tm), PCR product length and chromosome location of SSLP (with respect to the RI map of May 1997, Weeds World 4i).

#### F. Production of hybrid plants

- 5 C24 plants heterozygous for *chlorina3A:Ac/GUS* are crossed as male to emasculated wildtype *Ler* to produce *Ler/C24(chlorina3A, GUS)* hybrid seeds.

Due to the heterozygosity of the C24 parent, only 50 % of hybrid plants have inherited the *chlorina3A:Ac/GUS* locus. The remaining 50% of hybrid plants are wildtype with respect to *chlorina3A:Ac/GUS*. Since the mutant locus is linked to a kanamycin  
10 resistance gene (contained on the same T-DNA as *GUS*) mutant plants can be pre-selected by germinating hybrid seeds on germination medium containing 50 mg/L kanamycin.

*Ler* plants homozygous for the five chromosome markers are male sterile (*ms1-1*) and are crossed without emasculation to wildtype C24 to produce *Ler(an-1, py-1, gl1-1, cer2-1, ms1-1)/C24* hybrid seeds.

- 15 Other *Ler* plants, which are male fertile, are crossed after emasculation of the female parent to produce *Ler/C24* hybrid seeds.

#### G. Introduction of *MSH3* and *MSH6/3* antisense genes into *Arabidopsis* and analysis of meiotic homeologous recombination

##### (i) Transformation of hybrid plants and analysis of homeologous meiotic recombination

- 20 The plant transformation vectors comprising the antisense genes described in (B) and (C) above are introduced into *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991, Bio/Technology 9, 963-967) by electroporation. Recombinant *Agrobacterium* clones are selected on LB medium containing 50 mg/L rifampicin and 100 mg/L carbenicillin. Selected clones are used to infect roots of *Arabidopsis* hybrid plants (described in (F)  
25 above) using the root transformation protocol of Valvekens et al. (1988, PNAS 85, 5536-5540) except that shoot and root inducing media contain hygromycin (10 mg/L) instead of kanamycin.

Plants regenerated from roots of hybrid plants are genetic clones of root donating plants and therefore are again genetic hybrids of two *Arabidopsis* subspecies described in  
30 (F). However, in contrast to the root donating plants, the regenerated hybrid plants also contain the introduced transgene and the co-introduced hygromycin resistance gene with the latter allowing these plants to grow on hygromycin containing culture medium.

Hygromycin resistant plants are then allowed to enter the reproductive phase and to produce gametes by meiotic divisions of microspore and megaspore mothercells. At  
35 meiosis, the *DMC1* promoter is activated and can direct the expression of antisense genes described in (B) and (C) above, leading to decreased *MSH6* and/or *MSH3* gene

expression. This in turn depletes the gamete mothercells of MSH6 and/or MSH3 protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between homeologous chromosomes.

Transgenic plants are then allowed to self-fertilise and to produce seeds. These 5 seeds (F2 seeds with respect to hybrid production), and the plants derived therefrom, carry the homeologous recombination events which can be identified by using the visual and molecular recombination markers described in (E) above.

In case of homeologous recombination between chromosomes of *Ler* and C24(*chlorina3A:Ac, GUS*), the analysis concentrates on chromosome 2 by selecting plants 10 showing the visual phenotypic marker *chlorina*. This marker thus serves as a reference point as it indicates that respective chromosomes 2 originate from C24. Other markers, such as *GUS* or molecular markers, on chromosome 2 may then be used to identify chromosomal regions which are derived from the *Ler* chromosome as a result of homeologous recombination. F2 plants of control transformants not expressing the 15 antisense gene(s) can be analysed in parallel and the results can be used for comparison to homeologous recombination results obtained in antisense plants.

(ii) Transformation of C24 wildtype, hybrid plant production and analysis of homeologous meiotic recombination

Introduction of MMR altering genes into wildtype C24 is done using the root 20 transformation protocol as described in G(i) for transformation of hybrid plants. Transformed plants are selected by resistance to either 10 mg/L hygromycin (in case of transformation with T-DNA's derived from pNOS-Hyg-SCV) or to 50 mg/L kanamycin (in case of transformation with T-DNA's derived from pBIN19).

Transgenic plants are then allowed to self-fertilise and to produce seeds (T1 seeds). 25 Segregation of the antibiotic resistance gene in the T1 population then indicates the number of transgene loci. Lines with a single transgene locus (indicated by a 3:1 ratio of resistant:sensitive plants) are selected and are bred to homozygosity. This is done by collecting selfed seeds (T2) from T1 plants and subsequent testing of at least four independent T2 seed populations for segregation of the antibiotic resistance gene. T2 30 populations which do not segregate the antibiotic resistance gene are assumed to be homozygous for both the resistance gene and the linked MMR altering gene.

C24 plants homozygous for the MMR altering gene are then crossed to *Ler* lines homozygous for recessive visual markers (see E(ii)) to produce C24/*Ler* hybrid plants as described in (F). These F1 hybrids are then allowed to enter the reproductive phase and to 35 produce gametes by meiotic division of microspore and megaspore mothercells. At meiosis, the *DMC 1* promoter is activated and can direct the expression of antisense or sense genes described in (B), (C) and (D) above, leading to decreased *MSH6* and/or *MSH3* gene expression. This in turn depletes the gamete mothercells of *MSH6* and/or *MSH3*

protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between the homeologous chromosomes of *C24* and *Ler*. Recombination events are then scored in the F2 generation.

For recombination analysis, the hybrid plants are allowed to self-fertilise and to produce F2 seeds. F2 plants are then preselected for a first visual marker. Since this marker is recessive, its visual presence indicates homozygosity for *Ler* DNA at the relevant locus. Those F2 plants which show this first visual marker are then analysed for the presence or absence of a second visual marker which in the *Ler* parent is closely linked to the first marker. Absence of the second visual marker indicates recombination between the relevant *C24* and *Ler* chromosomes between the first and second marker. The frequency of recombination in transgenic hybrids is compared to the recombination frequency in control hybrids not expressing the MMR altering gene.

Examples of recombination analysis are the following.

The *Ler* line NW22(*dis1*, *ga4*, *th1*) is used for crosses to transformed *C24*.

F2 plants are preselected first for thiamine requirement (*th1*) and then are further analysed for re-appearance of wildtype height (loss of *ga4*) and/or re-appearance of normal trichomes (loss of *dis1*) as a result of recombination.

The *Ler* line NW10(*tz-201*, *cer3*) is used for crosses to transformed *C24*.

F2 plants are then preselected first for thiazole requirement (*tz*) and then are further analysed for re-appearance of normal, i.e. non-shiny stems (loss of *cer3*) as a result of recombination.

The *Ler* line NW134 (*ttg*, *ga3*) is used for crosses to transformed *C24*. F2 plants are first preselected for dwarfish appearance (*ga3*) and are then analysed for re-appearance of trichomes (loss of *ttg*) as a result of recombination.

*Ler* plants homozygous for *abi3-1* and *gll-1* are used for crosses to transformed *C24*. F2 plants are first preselected for their ability to germinate in the presence of abscisic acid and are then analysed for re-appearance of trichomes on the leaves (loss of *gll-1*) as a result of recombination.

In the case of homeologous recombination between transformed *C24* and the *Ler* line NW1 (*an-1*, *py-1*, *gll-1*, *cer2-1*, *msl-1*), recombination analysis is similar the one described above, except that the second marker is not a visual marker but has to be a molecular marker. This is because the *Ler* parent carries only one visual marker per chromosome.

TABLE 1: SSLP Markers in *Arabidopsis thaliana* Subspecies

Chromosome	RI Map Position	PCR Primer Pair	Primer Sequence	T <sub>m</sub> [°C]	length/COL	length/LER	length/C24
I	2.3	ATEAT1 F ATEAT1 R	GCCACTGCGTGAATGATATG CGAACAGCCAACATTATCCCC	57.8 58.2	172	162	162
I	9.3	NGA63 F NGA63 R	AACCAAGGCACAGAAAGCG ACCCAAGTGATCGCCACC	57.3 59.6	111	89	120
I	40.1	NGA248 F NGA248 R	TACCGAAACCAAAACACAAAGG TCTGTATCTCGGTGAATTCTCC	56.1 58.2	143	129	no amplific.
I	81.2	NGA128 F NGA128 R	GGTCTGTTGATGTCGTAAGTCG ATCTTGAAACCTTTAGGGAGGG	60.1 58.2	180	190	no amplific.
I	81.2	NGA280 F NGA280 R	CTGATCTCACGGACAATAGTGC GGCTCCATAAAAAAGTGCACC	60.1 57.8	105	85	85
I	111.4	NGA111 F NGA111 R	CTCCAGTTGGAAAGCTAAAGGG TGTTTTTTTAGGACAAATGGCG	60 70	128	162	170
II	ca. 7.5	NGA168 F NGA168 R	CCTTCACATCCAAAACCCAC GCACATACCCACAACCCAGAA	57.8 57.8	213	217	208

II	ca. 48	NGA1126L	CGCTACGCTTTTCGGTAAAG	57.8	191	199	196
		NGA1126R	GCACAGTCCAAGTCACAACC	59.9			
II	62.2	NGA361L	AAAGAGATGAGAATTGGAC	51.7	114	120	114
		NGA361R	ACATATCAATATATTAAAGTAGC	49.5			
II	73	NGA168 F	TCGTCTACTGCACCTGCCG	59.6	151	135	135
		NGA168 R	GAGGACATGTATAGGAGCTCG	61.9			
II	ca. 77	AthBIO2 L	TGACCTCCTCTTCCATGGAG	59.9	141	209	139
		AthBIO2 R	TTAACAGAAACCCAAAGCTTTC	54.5			
II	ca. 83	AthUBIQUE L	AGGCAAATGTCCATTTCATTG	54.1	146	148	148
		AthUBIQUE R	ACGACATGGCAGATTCTCC	57.8			
III	3.4	NGA172 F	AGCTGCTTCCTTATAGCGTCC	60	162	136	140
		NGA172 R	CATCCGAATGCCATTGTTC	55.4			
III	12.8	NGA126 F	GAAAAACGCTACTTTCGTGG	56.1	119	147	no amplif.
		NGA126 R	CAAGAGCAATATCAAGAGCAGC	58.2			
III	17.5	NGA162 F	CATGCAATTGTCATCTGAGG	55.8	107	89	no amplif.
		NGA162 R	CTCTGTCACTCTTTTCCTCTGG	60.1			

III	81.8	NGA6 F	TGGAATTCCTCCTCTCTC	56.1	143	123	143
		NGA6 R	ATGGAGAAGCTTACACTGATC	56.1			
IV	19.8	NGA12 F	AATGTTGCTCCTCCCTCCTC	59.9	247	234	220
		NGA12 R	TGATGCTCTCTGAAACAAGAGC	58.2			
IV	24.1	NGA8 F	GAGGCAAAATCTTTATTTCCG	56.1	154	198	190
		NGA8 R	TGGCTTTCGTTTATAAACATCC	54.5			
IV	102	NGA1107 L	GCGAAAAAACAAAAAATCCA	50.2	150	140	140
		NGA1107 R	CGACGAATCGACAGAAATTAGG	58			
V	11.8	NGA225 F	GAAATCCAAATCCCAGAGAGG	58	119	189	119
		NGA225 R	TCTCCCCACTAGTTTGTGTC	60.1			
V	16.7	NGA249 F	TACCGTCAATTTTCATCGCC	55.4	125	115	115
		NGA249 R	GGATCCCCTAACTGTAAAATCCC	58.2			
V	19.9	CA72 F	AATCCCAGTAACCAACACACA	56.3	124	110	110
		CA72 R	CCCAGTCTAACCCACGACCAC	61.9			
V	20	NGA151 F	GTTTGGGAAGTTTGTGCTGG	55.8	150	120	130
		NGA151 R	CAGTCTAAAAGCGAGAGTATGATG	58.6			

V	24	NGA106 F	GTTATGGAGTTTCTAGGGCACG	60.1	157	123	130
		NGA106 R	TGCCCCATTTTGTCTTCTC	55.8			
V	37.8	NGA139 F	AGAGCTACCAGATCCGATGG	59.9	174	132	132
		NGA139 R	GGTTTCGTTTCACTATCCAGG	55.8			
V	50	NGA76 F	GGAGAAAATGTCACCTCTCCACC	60.1	231	> 250	300
		NGA76 R	AGGCATGGGAGACATTTACG	57.8			
V	61.1	ATHSO191 L	CTCCACCAATCATGCAAAATG	55.8	148	156	146
		ATHSO191 R	TGATGTTGATGGAGATGGTCA	53.7			
V	81.7	NGA129 F	TCAGGAGGAACAAAGTGAGGG	60.1	177	179	172
		NGA129 R	CACACTGAAGATGGTCTTGAGG	60.1			

## CLAIMS

1. An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.
- 5 2. A DNA molecule according to claim 1 wherein said polypeptide is homologous to a mismatch repair polypeptide of a yeast or of a human.
3. A DNA molecule according to claim 1 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).
4. An isolated and purified polypeptide functionally involved in the DNA  
10 mismatch repair system of a plant.
5. A polypeptide according to claim 4 which is homologous to a mismatch repair polypeptide of a yeast or of a human.
6. An isolated and purified polypeptide selected from the group consisting of a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18), a polypeptide encoded by the  
15 gene *AtMSH6* (SEQ ID NO:30), polypeptides homologous to a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18) and polypeptides homologous to a polypeptide encoded by the gene *AtMSH6* (SEQ ID NO:30).
7. An isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is  
20 capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.
8. A DNA molecule according to claim 7 comprising a polynucleotide sequence  
25 encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence.
9. A DNA molecule according to claim 8 wherein said polynucleotide is capable  
30 of interfering with the expression of a plant polynucleotide sequence is a sense polynucleotide, an antisense polynucleotide or a ribozyme.
10. A DNA molecule according to claim 7 comprising a polynucleotide sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.



11. A DNA molecule according to claim 10 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).
12. A DNA molecule according to claim 10 further comprising a regulation element capable of causing overexpression of said polypeptide in a cell of said plant.
13. A chimeric gene comprising:
- 5 a DNA sequence selected from the group consisting of (i) a sequence encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a
- 10 sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant; and
- at least one regulation element capable of functioning in a plant cell.
14. A chimeric gene according to claim 13 wherein said regulation element is selected from constitutive, inducible, tissue type specific and cell type specific promoters.
- 15 15. A chimeric gene according to claim 13 comprising a DNA sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant, wherein said regulation element is capable of causing overexpression of said polypeptide in a cell of said plant.
16. A chimeric gene according to claim 13 wherein said regulation element is
- 20 selected from the group consisting of 35S, NOS, PR1a, AoPR1 and DMC1.
17. A plasmid or vector comprising a chimeric gene according to any one of claims 13-16.
18. A plant cell stably transformed, transfected or electroporated with a plasmid or vector according to claim 17.
- 25 19. A plant comprising a cell according to claim 18.
20. A plant according to claim 19 selected from plants of the families *Brassicaceae*, *Poaceae*, *Solanaceae*, *Asteraceae*, *Malvaceae*, *Fabaceae*, *Linaceae*, *Canabinaceae*, *Dauaceae* and *Cucurbitaceae*.
21. A process for at least partially inactivating a DNA mismatch repair system of a
- 30 plant cell, comprising transforming or transfecting said plant cell with a DNA molecule according to any one of claims 1-3 or 7-12 and causing said DNA sequence to express said polynucleotide or said polypeptide.
22. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a chimeric gene

according to any one of claims 13-16 and causing said DNA sequence to express said polynucleotide or said polypeptide.

23. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a plasmid or vector  
5 according to claim 17 and causing said DNA sequence to express said polynucleotide or said polypeptide.

24. A process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant;  
10 permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for plants in which homeologous recombination has occurred.

25. A process according to claim 24 wherein a first gene is incapacitated in said first plant, a second gene is incapacitated in said second plant, and said first and second genes are incapacitated in said hybrid plant thereby altering the mismatch repair system of  
15 said hybrid plant.

25. A process according to claim 25 wherein said incapacitation of the mismatch repair system of said hybrid plant is reversible.

26. A process according to claim 24 wherein a new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait is  
20 observable in at least one of said offspring plants.

27. A process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening  
25 said plants for a plant having said desired characteristic.

28. A process according to claim 27 wherein said step of altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene according to claim 13 and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a  
30 mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid plant, cell or cells.

29. A process according to claim 28 comprising inactivating an MSH3 gene and/or an MSH6 gene of said plant.

30. A process according to claim 28 comprising inactivating an MSH3 gene and an  
35 MSH6 gene of said plant.

31. A process according to claim 27 comprising at least partially inactivating the mismatch repair system of said plant in a predetermined cell type or in a predetermined tissue of said plant.

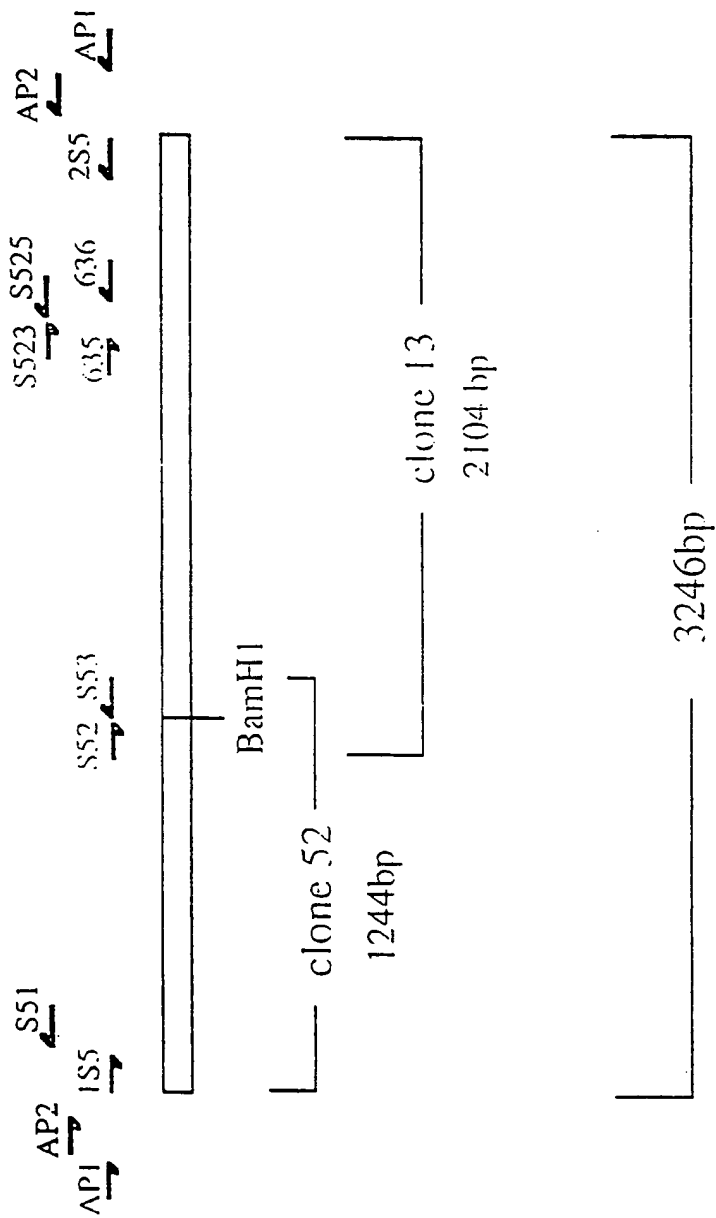
32. A process according to claim 31 further comprising restoring mismatch repair  
s in said cell type or said tissue.

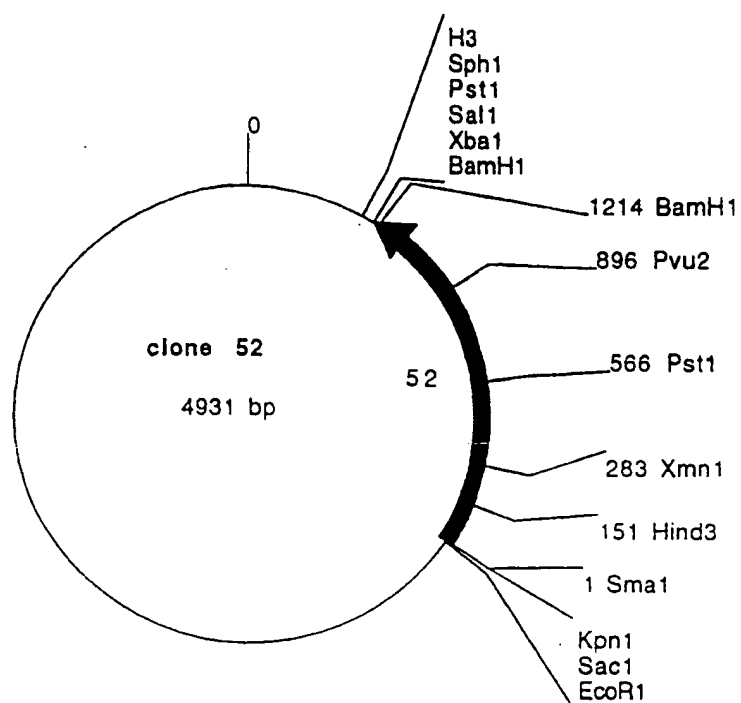
33. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule according to claim 1 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

34. An oligonucleotide capable of hybridising at 45°C under standard PCR  
10 conditions to the DNA of SEQ ID NO: 18 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

35. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

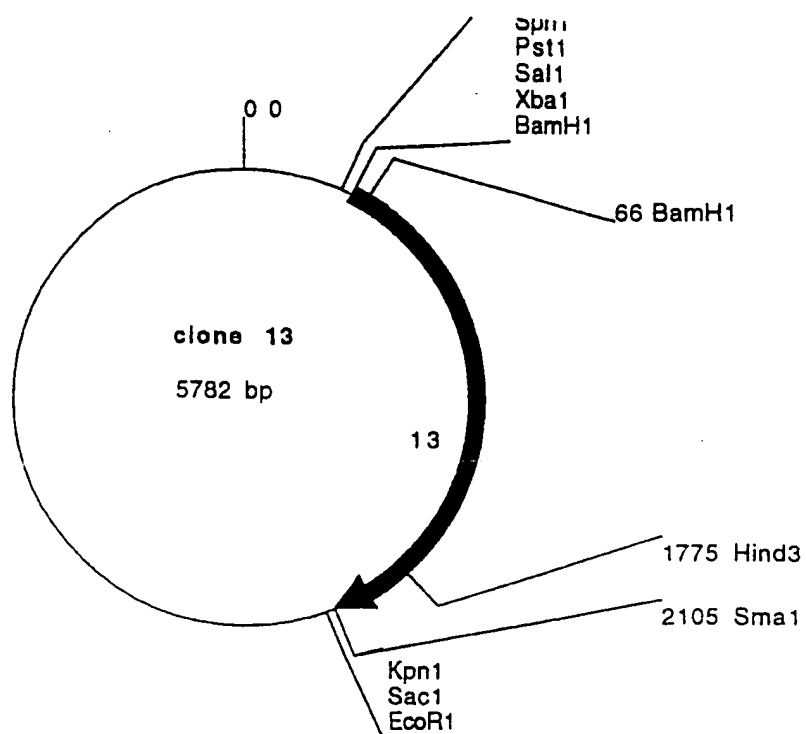
Figure 1





**Figure 2**

**Comments/References:** 52= 3' side of S5 (AtMSH3) 1244bp in pUC18/Sma1



**Figure 3**

**Comments/References:** 13 = 3' side of S5 (A1MSH3) 2104bp in pUC18/SmaI

1 cCTAAGAAAGCGCGGAAAATTGGCAACCCCAAGTTGCGCATAGCCACGACACGACCTTCCATTTCCTCTTAACGGAGGA 80  
 81 GATTACGAATAAAGCAATT ATG GGC AAG CAA AAG CAG CAG ACQ ATT TCT CGT TTC TTT GCT CCC 144  
 1 M G K Q K Q Q T I S R F A P 15  
 145 AAA CCC AAA TCC CCG ACT CAC GAA CCG AAT CCG GTA GCC GAA TCA ACA CCG CCA CCG 204  
 16 K P K S P T H E P N P V A E S S T P P P 35  
 205 AAG ATA TCC GCC ACT GTA TCC TTT TCT CCT TCC AAG CGT AAG CTT CTC TCC GAC CAC CTC 264  
 36 K I S A T V S F S P S K R K L L S D H L 55  
 265 GCC GCC GCG TCA CCC AAA AAG CCT AAA CTT TCT CCT CAC ACT CAA AAC CCA GTA CCC GAT 324  
 56 A A A S P K K P K L S P H T Q N P V P D 75  
 325 CCC AAT TTA CAC CAA AGA TTT CTC CAG AGA TTT CTG GAA CCC TCG CCG GAG GAA TAT GTT 384  
 76 P N L H Q R F L Q R F L E P S P E Y V 95  
 385 CCC GAA ACG TCA TCA TCG AGG AAA TAC ACA CCA TTG GAA CAG CAA GTG GTG GAG CTA AAG 444  
 96 P E T S S S R K Y T P L E Q Q V V E L K 115  
 445 AGC AAG TAC CCA GAT GTG GTT TTG ATG GTG GAA GTT GGT TAC AGG TAC AGA TTC TTC GGA 504  
 116 S K Y P D V V L M V E V G Y R Y R F F G 135  
 505 GAA GAC GCG GAG ATC GCA GCA CGC GTG TTG GGT ATT TAC GCT CAT ATG GAT CAC AAT TTC 564  
 136 E D A E I A A R V L G I Y A H M D H N F 155  
 565 ATG ACG GCG AGT GTG CCA ACA TTT CGA TTG AAT TTC CAT GTG AGA AGA CTG GTG AAT GCA 624  
 156 M T A S V P T F R L N F H V R L V N A 175  
 625 GGA TAC AAG ATT GGT GTA GTG AAG CAG ACT GAA ACT GCA GCC ATT AAG TCC CAT GGT GCA 684  
 176 G Y K I G V V K Q T E T A A I K S H G A 195  
 665 AAC CGG ACC GGC CCT TTT TTC CGG GCA CTG TCG GCG TTG TAT ACC AAA GCC ACG CTT GAA 744  
 196 N R T G P F F R G L S A L Y T K A T L E 215  
 745 GCG GCT GAG GAT ATA AGT GGT GGT TGT GGT GAA GAA GGT TTT GGT TCA CAG AGT AAT 804  
 216 A A E D I S G G C G G E E G F G S Q S N 235  
 805 TTC TTG GTT TGT GTT GAT GAG AGA GTT AAG TCG GAG ACA TTA GGC TGT GGT ATT GAA 864  
 236 F L V C V V D E R V K S E T L G C G I E 255  
 865 ATG AGT TTT GAT GTT AGA GTC GGT GTT GGC GTT GAA ATT TCG ACA GGT GAA GTT GTT 924  
 256 M S F D V R V G V V G V E I S T G E V V 275

Figure 4

925	TAT	GAA	GAG	TTC	AAT	GAT	AAT	TTC	ATG	AGA	AGT	GGA	TTA	GAG	GCT	GTG	ATT	TTG	AGC	TTG	984
276	Y	E	E	F	N	D	N	F	M	R	S	G	L	E	A	V	I	L	S	L	295
985	TCA	CCA	GCT	GAG	CTG	TTG	CTT	GGC	CAG	CCT	CTT	TCA	CAA	CAA	ACT	GAG	AAG	TTT	TTG	GTG	1044
296	S	P	A	E	L	L	L	G	Q	P	L	S	Q	Q	T	E	K	F	L	V	315
1045	GCA	CAT	GCT	GGA	CCT	ACC	TCA	AAC	GTT	CGA	GTG	GAA	CGT	GCC	TCA	CTG	GAT	TGT	TTC	AGC	1104
316	A	M	A	G	P	T	S	N	V	R	V	E	R	A	S	L	D	C	F	S	335
1105	AAT	GGT	AAT	GCA	GTA	GAT	GAG	GTT	ATT	TCA	TTA	TGT	GAA	AAA	ATC	AGC	GCA	GGT	AAC	TTA	1164
336	N	G	N	A	V	D	E	V	I	S	L	C	E	K	I	S	A	G	N	L	355
1165	GAA	GAT	GAT	AAA	GAA	ATG	AAG	CTG	GAG	GCT	GCT	GAA	AAA	GGA	ATG	TCT	TGC	TTG	ACA	GTT	1224
356	E	D	D	K	E	M	K	L	E	A	A	E	K	G	M	S	C	L	T	V	375
1225	CAT	ACA	ATT	ATG	AAC	ATG	CCA	CAT	CTG	ACT	GTT	CAA	GCC	CTC	CTA	ACG	TTT	TGC	CAT		1284
376	H	T	I	M	N	M	P	H	L	T	V	Q	A	L	A	L	T	F	C	H	395
1285	CTC	AAA	CAG	TTT	GGA	TTT	GAA	AGG	ATC	CTT	TAC	CAA	GGG	GCC	TCA	TTT	CGC	TCT	TTG	TCA	1344
396	L	K	Q	F	G	F	E	R	I	L	Y	Q	G	A	S	F	R	S	L	S	415
1345	AGT	AAC	ACA	GAG	ATG	ACT	CTC	TCA	GCC	AAT	ACT	CTG	CAA	CAG	TTG	GAG	GTT	GTG	AAA	AAT	1404
416	S	N	T	E	M	T	L	S	A	N	T	L	Q	Q	L	E	V	V	K	N	435
1405	AAT	TCA	GAT	GGA	TCG	GAA	TCT	GGC	TCC	TTA	TTC	CAT	AAT	ATG	AAT	CAC	ACA	CTT	ACA	GTA	1464
436	N	S	D	G	S	E	S	G	S	L	F	H	N	M	N	H	T	L	T	V	455
1465	TAT	GCT	TCC	AGG	CTT	CTT	AGA	CAC	TGG	GTG	ACT	CAT	CCT	CTA	TGC	GAT	AGA	AAT	TTG	ATA	1524
456	Y	G	S	R	L	L	R	H	W	V	T	H	P	L	C	D	R	N	L	I	475
1525	TCT	GCT	CGG	CTT	GAT	GCT	GTT	TCT	GAG	ATT	TCT	GCT	TGC	ATG	GGA	TCT	CAT	AGT	TCT	TCC	1584
476	S	A	R	L	D	A	V	S	E	I	S	A	C	M	G	S	H	S	S	S	495
1585	CAG	CTC	AGC	AGT	GAG	TTG	GTT	GAA	GAA	GGT	TCT	GAG	AGA	GCA	ATT	GTA	TCA	CCT	GAG	TTT	1644
496	Q	L	S	S	E	L	V	E	E	G	S	E	R	A	I	V	S	P	E	F	515
1645	TAT	CTC	GTG	CTC	TCC	TCA	GTC	TTG	ACA	GCT	ATG	TCT	AGA	TCA	TCT	GAT	ATT	CAA	CGT	GGA	1704
516	Y	L	V	L	S	S	V	L	T	A	M	S	R	S	S	D	I	Q	R	G	535
1705	ATA	ACA	AGA	ATC	TTT	CAT	CGG	ACT	GCT	AAA	GCC	ACA	GAG	TTC	ATT	GCA	GTT	ATG	GAA	GCT	1764
536	I	T	R	I	F	H	R	T	A	K	A	T	E	F	I	A	V	M	E	A	555
1765	ATT	TTA	CTT	CGG	GGG	AAG	CAA	ATT	CAG	CGG	CTT	GGC	ATA	AAG	CAA	GAC	TCT	GAA	ATG	AGG	1824
556	I	L	L	A	G	K	Q	I	Q	R	L	G	I	K	Q	D	S	E	M	R	575

Figure 4 (Continued)



1825 576	AGT S	ATG M	CAA Q	TCT S	GCA A	ACT T	GTG V	CGA R	TCT S	ACT T	CTT L	TTG L	AGA R	AAA K	TTG L	ATT I	TCT S	GTT V	ATT I	TCA S
1885 596	TCC S	CCT P	GTT V	GTG V	GTT V	GAC D	AAT N	GCC A	GGG G	AAA K	CTT L	CTC L	TCT S	GCC A	CTA L	AAT N	AAG K	GAA E	GCG A	
1945 616	GTT V	CGA R	GGT G	GAC D	TTG L	CTC L	GAC D	ATA I	CTA L	ATC I	ACT T	TCC S	AGC S	GAC D	CAA Q	TTT F	CCT P	GAG E	GCT A	
2005 636	GAA E	GCT A	CGC R	CAA Q	GCA A	GTT V	TTA L	GTC V	ATC I	AGG R	GAA E	AAG K	CTG L	GAT D	TCC S	TCG S	ATA I	GCT A	TTT F	
2065 656	CGC R	AAG K	CTC L	GCT A	ATT I	CGA R	AAT N	TTG L	GAA E	TTT F	CTT L	CAA Q	GTG V	TCG S	GGG G	ATC I	ACA T	CAT H	TTG L	
2125 676	ATA I	GAG E	CTG L	CCC P	GTT V	GAT D	TCC S	AAG K	GTC V	CCT P	ATG H	AAT N	TGG W	GTG V	AAA K	GTA V	AAT N	AGC S	AAG K	
2185 696	AAG K	ACT T	ATT I	CGA R	TAT Y	CAT H	CCC P	CCA P	GAA E	ATA I	GTA V	GCT A	GGG G	TTG L	GAT D	GAG E	CTA L	GCT A	GCA A	
2245 716	ACT T	GAA E	CAT H	CTT L	GCC A	ATT I	GTG V	AAC N	CGA R	GCT A	TCG S	TGG W	GAT D	AGT S	TTC F	CTC L	AAG K	AGT S	AGT S	
2305 736	AGA R	TAC Y	TAC Y	ACA T	GAT T	TTT F	AAG K	GCT A	AAA V	GTT Q	CAA Q	GCT A	L A	GCA A	CTG L	GAC D	TGT C	TTG L	CAC H	
2365 756	TCC S	CTT L	TCA S	ACT T	CTA L	TCT S	AGA R	AAC N	AAG K	AAC N	TAT Y	GTC V	CGT R	CCC P	GAG E	TTT F	GTG V	GAT D	TGT C	
2425 776	GAA E	CCA P	GTT V	GAG E	ATA I	AAC N	ATA I	CAG Q	TCT S	GGT G	CGT R	CAT H	GTA P	CTG V	CTG L	GAG E	ACT T	ATA I	CAA Q	
2485 796	GAT D	AAC N	TTT F	GTG V	CCA P	AAT N	GAC D	ACA T	ATT I	TTG L	CAT H	GCA A	GAA E	GGG G	GAA E	TAT Y	TGC C	CAA Q	ATC I	
2545 816	ACC T	GGA G	CCT P	AAC N	ATG M	GGA G	GGA G	AAG K	AGC S	TGC C	TAT Y	ATC I	CGT R	CAA Q	GTT V	GCT A	TTA L	ATT I	ATA I	
2605 836	ATG M	GCT A	CAG Q	GTT V	GGT G	TCC S	TTT F	GTA V	CCA P	GCG A	TTC F	GCC A	AAG K	CTG L	CAC H	GTG V	CTT L	GAT D	GGT G	
2665 856	GTT V	TTC F	ACT T	CGG R	ATG M	GGT G	GCT A	TCA S	GAC D	AGT S	ATC I	CAG Q	CAT H	GGC G	AGA R	AGT S	ACC T	TTT F	GAA E	

Figure 4 (Continued)

2725 876	GAA TTA AGT GAA GCG TCA CAC ATA ATC AGA ACC TGT TCT TCT CGT TCG CTT GTT ATA TTA	2784 895
2785 896	GAT GAG CTT GGA AGA GGC ACT AGC ACA CAC GAC GGT GTA GCC ATT GCC TAT GCA ACA TTA	2844 915
2845 916	CAG CAT CTC CTA GCA GAA AAG AGA TGT TTG GTT CTT TTT GTC ACG CAT TAC CCT GAA ATA	2904 935
2905 936	GCT GAG ATC AGT AAC GGA TTC CCA GGT TCT GTT GGT GGT ACA TAC CAT GTC TCG TAT CTG ACA	2964 955
2965 956	TTG CAG AAG GAT AAA GGC AGT TAT GAT CAT GAT GAT GTG ACC TAC CTA TAT AAG CTT GTG	3024 975
3025 976	CGT GGT CTT TGC AGC AGG AGC TTT GGT TTT AAG GTT GCT CAG CTT GCC CAG ATA CCT CCA	3084 995
3085 996	TCA TGT ATA CGT CGA GCC ATT TCA ATG GCT GCA AAA TTG GAA GCT GAG GTA CGT GCA AGA	3144 1015
3145 1016	GAG AGA AAT ACA CGC ATG GGA GAA CCA GAA GGA CAT GAA GAA CCG AGA GGC GCA GAA GAA	3204 1035
3205 1036	TCT ATT TCG GCT CTA GGT GAC TTG TTT GCA GAC CTG AAA TTT GCT CTC TCT GAA GAG GAC	3264 1055
3265 1056	CCT TGG AAA GCA TTC GAG TTT TTA AAG CAT GCT TGG AAG ATT GCT GGC AAA ATC AGA CTA	3324 1075
3325 1076	AAA CCA ACT TGT TCA TTT TGA TTTAATCTTAACATTATAGCAACTGCNAGGTCTTGATCATCTCTAGTTGCG	3397 1082
3398 1	TACTAACTT ATG TGT ATT AGT ATA ACA AGA AAA GAG AAT TAG AGAG ATG GAT TCT AAT CCG	3458 5
3459 6	GTG TTG CAG TAC ATC TTT TCT CCA CCC GCA TAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	3522 16

Figure 4 (Continued)

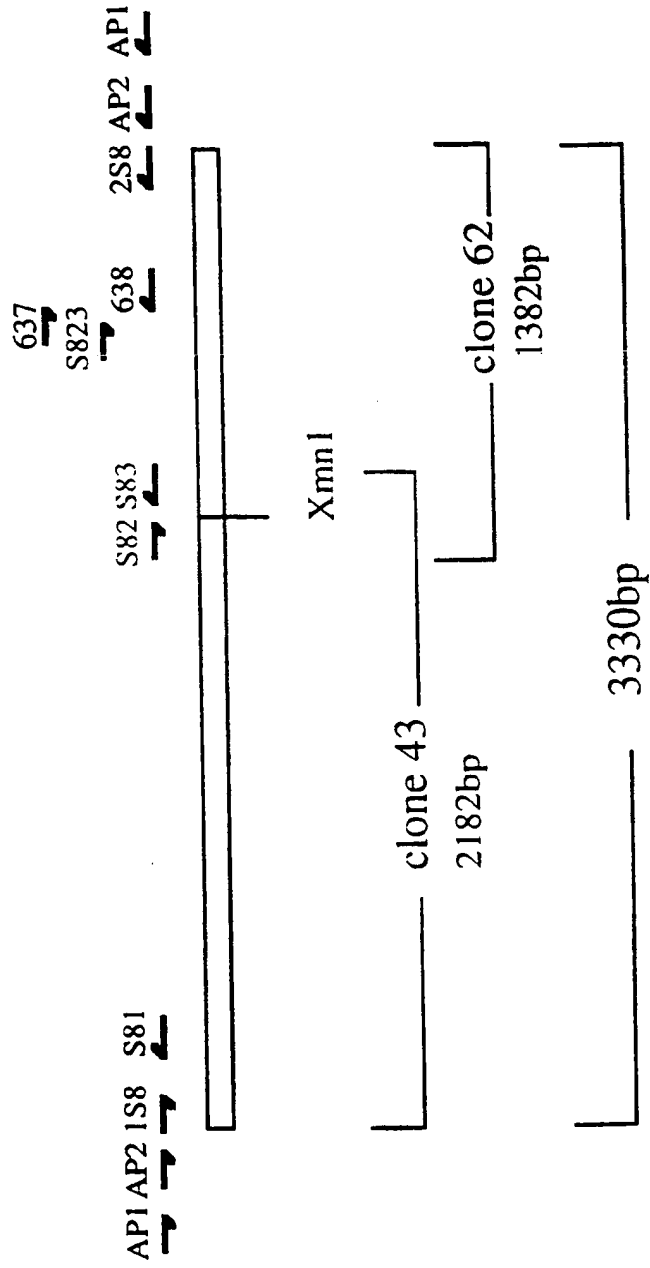
Figure 5

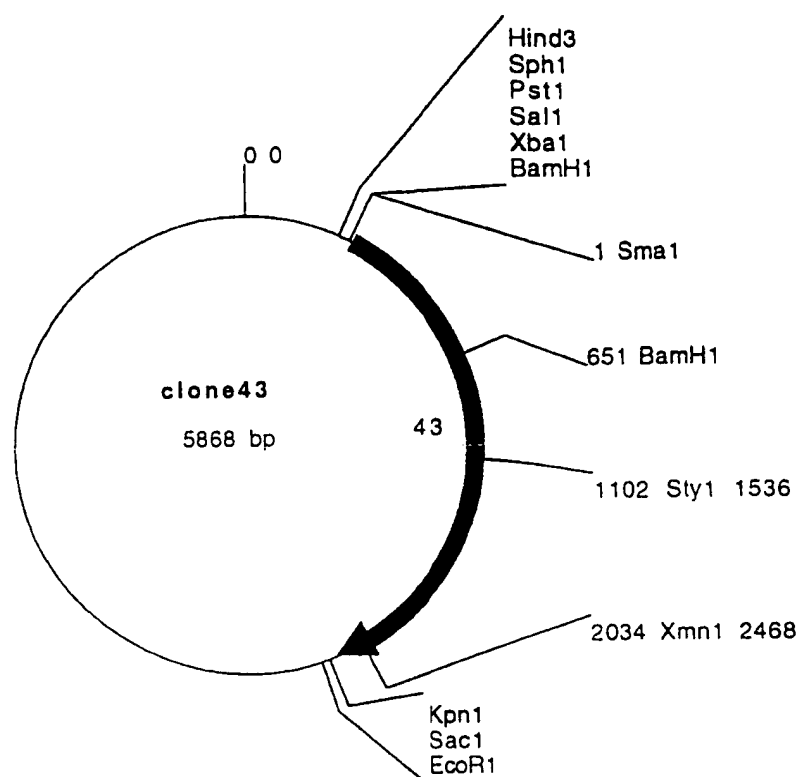
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1  --MCKQK-----QDISREFFAPKPKSP-CHZ-PNPFYASSTPPPK-----ISAVUSBSYKMKLL
1  MVIQNEPKLVLLRAKSSANRPI LLNLLTINAGQPTISRPFKKAVKSLCHHKQEQEVAVGMAQGSICLDTUEEDNLSSVABTPTVNDSPFLKUS
52  SDHLAAASPKEPKLQPHMQRPPVDPNLMHQRVLQVLEP-----SPZEVTPETSS---SRIFBLOQVMEKSYPOVZMVVGVY-PT-
56  VS9KNSKNSKKTSGTSTTPHDIQAKKLDRIKKKSDENVEADEDEEUGHOPVKKKAKKSPKALHEDKQVKDEKMMRDKZEVYINVGYPK-C
134  PORDREIAARVQUTYAH-----MDUN-----PMTASVPTFLRPVHARLVMAQYKIQVOKPTETAKKH-ETAMTQPPPGLSALYTKA
191  EAEADVTSRHEHKLVPKLTIDESNPQUCNHQPAYCEPPDOVRLNVHLELVHNLKVAVVEDAEESAKRHPDCAKSSVPERAKISMVPTKA
213  QLEAAEDISGOCGEOPGSSQMYLVCVYDERVKSETGCGIEMSPVUVVGVQVETISTDEVVYEEFMD-PMRSDOFAVILSPAPBTLG-UQ-
286  PPOVMSIFVLR-----GKR-----ILUDTNSIMASRDVHQGVAKYSLISVHLMSEVVYDEEERBLADKKQOIRHKYEQRIEVEVNTDU
306  CSQQTERKFLVAHAQPTSNRVVERASLDCYSNGNAVDVVSCEKISAGNLEDDKHKLEAAEKMSCLTVBTINMHPBLTVQALALTPCELKQPG
368  PPLHVAKEFKDISCELINKQYDLEDHVVQAIKVANAKQOSPEL-----INLVSKLYSHMVYEN
401  PERILYOGASPHSLSENTEHTTSAHTLQOQDEVVKNHSDGSEKSGSLFNNHNNHTLVYGSRLRRZVTPLCCDAMLGARDVSEISACMUSHSS
428  MEQVMLIPSIYSPPASEKIHLEOPNSLOSLDIYTHD--GUK-GSEPLMLDHTNISPTGLRWRERAILKPLVDVHQMERLDAIECTTSEINNS---
496  QLSSELVREOSRAIVSPEYLVLSSEVLTAMSHSSSIOGQIITRIFRHTAKATEPIAVMBAJLLAQKQIQRLQIKQDSMMMSQS-ATVMSTCLRM
517  -----IPFESLQNLNHTPDLBTLMRIYQCTSKKEVITYLKOHTSPVDHYKMQBSILSEHFMSSDORIUQSPLELFR
590  SISVISPVVVDMAOKLCSAATKKAIVRG-----DLCDILITS-SOPEPLAEARQAVLVIRKEDSSIASPTKKIATIMMCEQLVSGITHLIELP
591  PPSLNNELLSTTOLPHELTNIVSVNMEKNSDKQVBPZNLNNYDCSEGIKIQRCESESVRSQKESELATIRKYEXRPYEMERDEVDILIEVANS
680  VDSKVPMMNVRVNSFKKTIYVPEEIVAGLDEKALATERHAIVNKASWDSLSKSPRYTQDFKAAVOAQAALSCLEHESSTLSRMRKYVRPSPVDD
686  QIKDLEDDMIKVRNFKMVSRTTERTOKLTQKLEYXKDLQIKSELYKEPLENKITAEVTLKKITLNEAQYDCHLSLAAATSCNRYVRPSPVDD
775  CEPVEINIQSQRHPVLETIFQDNTVPHDTILHAEGEYCQITGPHHGGKSCYIROVALTQHAQVSGSPVAPASFAEMVLDQVPTFMGASSSQMG
781  QQ--AIAKANAMPDIES-LOVHYVENDIMMSPENGKINHTTGPHHGGKESYIROVALTQHAQVSGSPVAPASFAEMVLDQVPTFMGASSSQMG
870  NSTELELSRASHIITTCSSRSVJDEDELGRGTSRHDGVNATKATLQHLAEKRC-LVPEVYVHTPTETIAEISNGPFGSVQTYHVASLTLOKDKSY
873  DETSKVEMLDILHILEKNCNRSELLEDEVGROTPJTHDGIATSAVALIKYFSELSDCPELLETHPTMLOGEIKS---PLIMMHMDVVEROK--TGE
964  QMDDTYTAKVVGELCSRSYQPKVQAQVQIIPSCIRNAISMAATQASVMAARENTHMOPECHNEPRGAEESISACQDLFAQKPTFMKRUHMK
963  QMNSYIYKSLKAGETYNISGMNVAKLRLDKDILMRAPYSISETEKESIN-----EOLAKB---ESSEKRIKSDH---
MSH3_AT 1059 APEFLKXHWKAAKIRKPTCTSP---
MSH3_SC 1032 -----PTATDKAKLLSLDIH

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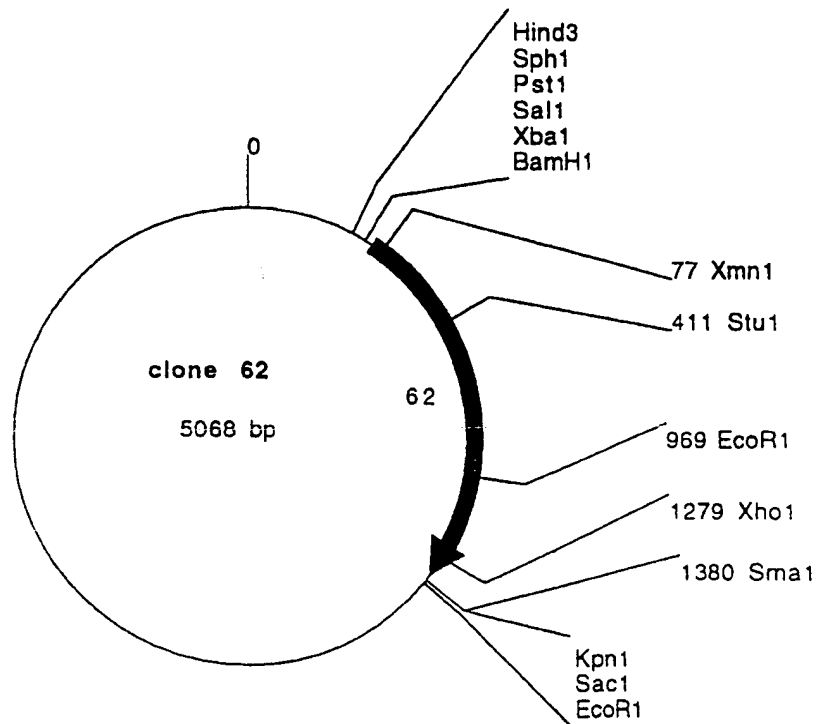
Figure 6





**Figure 7**

**Comments/References:** 43= 5' side of S8 (AIMSH6) 2182 bp in pUC18/Sma1



**Figure 8**

**Comments/References:** 62= 3' side of S8 (AtMSH6) 1379bp in pUC18/Sma1

1 AAAAGTTGAGCCCTGAGGAGTATCGTTTCCGCCATTCTACGACGAAGCGGAAAAATTTTGGCGCAATCTTTCCCCCC 80  
 81 TTTTCGAATTCTCTCAGCTCAAAACATCGTTTCTCTCTCACTCTCTCTCACAATTCACAAAAA ATG CAG CGC CAG 153  
 1 TTTTCGAATTCTCTCAGCTCAAAACATCGTTTCTCTCTCACTCTCTCTCACAATTCACAAAAA M Q R Q 4  
 154 AGA TCG ATT TTG TCT TTC CAA AAA CCC ACC GCG GCG ACT ACG AAG GGT TTG GTT TCC 213  
 1 R S I L S F F Q K P T A A T T K G L V S 24  
 214 GGC GAT GCT GCT AGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC 273  
 25 G D A A S G 44  
 274 GAT GCT AAA GGC GAC GCT TCT GTA CGT TTT GCT GTT TCG AAA TCT GTC GAT GAG GTT AGA 333  
 45 D A K G D A S V R F A V S K S V D E V R 64  
 334 GGA ACG GAT ACT CCA CCG GAG AAG GTT CCG CGT CGT GTC CTG CCG TCT GGA TTT AAG CCG 393  
 65 G T D T P P E K V P R R V L P S G F K P 84  
 394 GCT GAA TCC GCC GST GAT GCT TCG TCC CTG TTC TCC AAT ATT ATG CAT AAG TTT GTA AAA 453  
 85 A E S A G D A S S L F S N I M H K F V K 104  
 454 GTC GAT GAT CGA GAT TGT TCT GGA GAG AGG AGC CGA GAA GAT GTT CCG CTG AAT GAT 513  
 105 V D D R D C S S G E R S R E D V V P L N D 124  
 514 TCA TCT CTA TGT ATG AAG GCT AAT GAT GTT ATT CCT CAA TTT CGT TCC AAT AAT GGT AAA 573  
 125 S S L C M K A N D V I P Q F R S N N G K 144  
 574 ACT CAA GAA AGA AAC CAT GCT TTT AGT TTC AGT GGG AGA GCT GAA CTT AGA TCA GTA GAA 633  
 145 T Q E R N H A F S F S G R A E L R S V E 164  
 634 GAT ATA GGA GTA GAT GGC GAT GTT CCT GGT CCA GAA ACA CCA GGG ATG CGT CCA CGT GCT 693  
 165 D I G V D G D V P G P E T P G M R P R A 184  
 694 TCT CGC TTG AAG CGA GTT CTG GAG GAT GAA ATG ACT TTT AAG GAG GAT AAG GTT CCT GTA 753  
 185 S R L K R V L E D E M T F K E D K V P V 204  
 754 TTG GAC TCT AAC AAA AGG CTG AAA ATG CTC CAG GAT CCG GTT TGT GGA GAG AAG AAA GAA 813  
 205 L D S N K R L K M L Q D P V C G E K K E 224  
 814 GTA AAC GAA GGA ACC AAA TTT GAA TGG CTT GAG TCT TCT CGA ATC AGG GAT GCC AAT AGA 873  
 225 V N E G T K F E W L E S S R I R D A N R 244  
 874 AGA CGT CCT GAT GAT CCC CTT TAC GAT AGA AAG ACC TTA CAC ATA CCA CCT GAT GTT TTC 933  
 245 R R P D D P L Y D R K T L H I P P D V F 264

Figure 9

934	AAG AAA ATG TCT GCA TCA CAA AAG CAA TAT TGG AGT GTT AAG AGT GAA TAT ATG GAC ATT	993
265	K K M S A S Q K Q Y W S V K S E Y M D I	284
996	GTG CTT TTC TTT AAA GTG GGG AAA TTT TAT GAG CTG TAT GAG CTA GAT GCG GAA TTA GGT	1053
285	V L F F K V G K F Y E L Y E L D A E L G	304
1054	CAC AAG GAG CTT GAC TGG AAG ATG ACC ATG AGT GGT GTG GGA AAA TGC AGA CAG GTT GGT	1113
305	H K E L D W K M T M S G V G K C R Q V G	324
1114	ATC TCT GAA AGT GGG ATA GAT GAG GCA GTG CAA AAG CTA TTA GCT CGT GGA TAT AAA GTT	1173
325	I S E S G I D E A V Q K L L A R G Y K V	344
1174	GGA CGA ATC GAG CAG CTA GAA ACA TCT GAC CAA GCA AAA GCC AGA GGT GCT AAT ACT ATA	1233
345	G R I E Q L E T S D Q A K A R G A N T I	364
1234	ATT CCA AGG AAG CTA GTT CAG GTA TTA ACT CCA TCA ACA GCA AGC GAG GGA AAC ATC GGG	1293
365	I P R K L V Q V L T P S T A S E G N I G	384
1294	CCT GAT GCC GTC CAT CTT GCT ATA AAA GAG ATC AAA ATG GAG CTA CAA AAG TGT TCA	1353
385	P D A V H L L A I K E I K M E L Q K C S	404
1354	ACT GTG TAT GGA TTT GCT TTT GAT TGT GCT GCC TTG AGG TTT TGG GTT GGG TCC ATC	1413
405	T V Y G F A F V D C A A L R F W V G S I	424
1414	AGC GAT GAT GCA TCA TGT GCT CTT GGA GCG TTA TTG ATG CAG GTT TCT CCA AAG GAA	1473
425	S D D A S C A A L G A L L M Q V S P K E	444
1474	GTG TTA TAT GAC AGT AAA GGG CTA TCA ACA GAA GCA CAA AAG GCT CTA AGG AAA TAT ACG	1533
445	V L Y D S K G L S R E A Q K A L R K Y T	464
1534	TTG ACA GGG TCT ACG GCG GTA CAG TTG GCT CCA GTA CCA CAA GTA ATG GGG GAT ACA GAT	1593
465	L T G S T A V Q L A P V P Q V M G D T D	484
1594	GCT GCT GGA GTT AGA AAT ATA ATA GAA TCT AAC GGA TAC TTT AAA GGT TCT TCT GAA TCA	1653
485	A A G V R N I I E S N G Y F K G S S E S	504
1654	TGG AAC TGT GCT GTT GAT GGT CTA AAT GAA TGT GAT GTT GCC CTT AGT GCT CTT GGA GAG	1713
505	W N C A V D G L N E C D V A L S A L G E	524
1714	CTA ATT AAT CAT CTG TCT AGG CTA AAG CTA AAG GAT GTA CTT AAG CAT GGG GAT ATT TTT	1773
525	L I N H L S R L K L E D V L K H G D I F	544
1774	CCA TAC CAA GTT TAC AGG GGT TGT CTC AGA ATT GAT GGC CAG ACG ATG GTA AAT CTT GAG	1833
545	P Y Q V Y R G C L R I D G Q T M V N L E	564

Figure 9 (Continued)



1834	ATA	TTT	AAC	AAT	AGC	TGT	GAT	GGT	GGT	CCT	TCA	GGG	ACC	TTG	TAC	AAA	TAT	CTT	GAT	AAC	1893
565	I	F	N	N	S	C	D	G	G	P	S	G	T	L	Y	K	Y	L	D	N	584
1894	TGT	GTT	AGT	CCA	ACT	GGT	AAG	CGA	CTC	TTA	AGG	AAT	TGG	ATC	TGC	CAT	CCA	CTC	AAA	GAT	1953
585	C	V	S	P	T	G	K	R	L	L	R	N	W	I	C	H	P	L	K	D	604
1954	GTA	GAA	AGC	ATC	AAT	AAA	CGG	CTT	GAT	GTA	GTT	GAA	GAA	TTC	ACG	GCA	AAC	TCA	GAA	AGT	2013
605	V	E	S	I	N	K	R	L	D	V	V	E	E	F	T	A	N	S	E	S	624
2014	ATG	CAA	ATC	ACT	GGC	CAG	TAT	CTC	CAC	AAA	CTT	CCA	GAC	TTA	GAA	AGA	CTG	CTC	GGA	CGC	2073
625	M	Q	I	T	G	Q	Y	L	H	K	L	P	D	L	E	R	L	L	G	R	644
2074	ATC	AAG	TCT	AGC	GTT	CGA	TCA	TCA	GCC	TCT	GTG	TTG	CCT	GCT	CTT	CTG	GGG	AAA	AAA	GTG	2133
645	I	K	S	S	V	R	S	S	A	S	V	L	P	A	L	L	G	K	K	V	664
2134	CTG	AAA	CAA	CGA	GTT	AAA	GCA	TTT	GGG	CAA	ATT	CTG	AAA	GGG	TTT	AGA	AGT	GGA	ATT	GAT	2193
665	L	K	Q	R	V	K	A	F	G	Q	I	V	K	G	F	R	S	G	I	D	684
2194	CTG	TTG	TTG	GCT	CTA	CAG	AAG	GAA	TCA	AAT	ATG	ATG	AGT	TTG	CTT	TAT	AAA	CTC	TGT	AAA	2253
685	L	L	A	L	A	Q	K	E	S	N	M	M	S	L	L	Y	K	L	C	K	704
2254	CTT	CCT	ATA	TTA	GTA	GGA	AAA	ACC	GGG	CTA	GAG	TTA	TTT	CTT	TCT	CAA	TTC	GAA	GCA	GCC	2313
705	L	P	I	L	V	G	K	S	G	L	E	L	F	L	S	Q	F	E	A	A	724
2314	ATA	GAT	AGC	GAC	TTT	CCA	AAT	TAT	CAG	AAC	CAA	GAT	GTG	ACA	GAT	GAA	AAC	GCT	GAA	ACT	2373
725	I	D	S	D	F	P	N	Y	Q	N	Q	D	V	T	D	E	N	A	E	T	744
2374	CTC	ACA	ATA	CTT	ATC	GAA	CTT	TTT	ATC	GAA	AGA	GCA	ACT	CAA	TGG	TCT	GAG	GTC	ATT	CAC	2433
745	L	T	I	L	I	E	L	F	I	E	R	A	T	Q	W	S	E	V	I	H	764
2434	ACC	ATA	AGC	TGC	CTA	GAT	GTC	CTG	AGA	TCT	TTT	GCA	ATC	GCA	GCA	AGT	CTC	TCT	GCT	GGA	2493
765	T	I	S	C	L	D	V	L	R	S	F	A	I	A	A	S	L	S	A	G	784
2494	AGC	ATG	GCC	AGG	CCT	GTT	ATT	TTT	CCC	GAA	TCA	GAA	GCT	ACA	GAT	CAG	AAT	CAG	AAA	ACA	2553
785	S	M	A	R	P	V	I	F	P	E	S	E	A	T	D	Q	N	Q	K	T	804
2554	AAA	GGG	CCA	ATA	CTT	AAA	ATC	CAA	GGA	CTA	TGG	CAT	CCA	TTT	GCA	GTT	GCA	GCC	GAT	GGT	2613
805	K	G	P	I	L	K	I	Q	G	L	W	H	P	F	A	V	A	A	D	G	824
2614	CAA	TTG	CCT	GTT	CCG	AAT	GAT	ATA	CTC	CTT	GGC	GAG	GCT	AGA	AGA	AGC	AGT	GGC	AGC	ATT	2673
825	Q	L	P	V	P	N	D	I	L	L	G	E	A	R	R	S	S	G	S	I	844
2674	CAT	CCT	CGG	TCA	TTG	TTA	CTG	ACG	GGA	CCA	AAC	ATG	GGC	GGA	AAA	TCA	ACT	CTT	CTT	CGT	2733
845	H	P	R	S	L	L	L	T	G	P	N	M	G	G	K	S	T	L	L	R	864

Figure 9 (Continued)

2734 865	GCA ACA TGT CTG GCC GTT ATC TTT GCC CAA CTT GGC TGC TAC GTG CCG TGT GAG TCT TGC	2793 884
2794 885	GAA ATC TCC CTC GTG GAT ACT ATC TTC ACA AGG CTT GGC GCA TCT GAT AGA ATC ATG ACA	2853 904
2854 905	GGA GAG AGT ACC TTT TTG GTA GAA TGC ACT GAG ACA GCG TCA GTT CTT CAG AAT GCA ACT	2913 924
2914 925	CAG GAT TCA CTA GTA ATC CTT GAC GAA CTG GGC AGA GGA ACT AGT ACT TTC GAT GGA TAC	2973 944
2974 945	GCC ATT GCA TAC TCG GTT TTT CGT CAC CTG GTA GAG AAA GTT CAA TGT CGG ATG CTC TTT	3033 964
3034 965	GCA ACA CAT TAC CAC CCT CTC ACC AAG GAA TTC GCG TCT CAC CCA CGT GTC ACC TCG AAA	3093 984
3094 985	CAC ATG GCT TGC GCA TTC AAA TCA AGA TCT GAT TAT CAA CCA CGT GGT TGT GAT CAA GAC	3153 1004
3154 1005	CTA GTG TTC TTG TAC CGT TTA ACC GAG GGA GCT TGT CCT GAG AGC TAC GGA CTT CAA GTG	3213 1024
3214 1025	GCA CTC ATG GCT GGA ATA CCA AAC CAA GTG GTT GAA ACA GCA TCA GGT GCT GCT CAA GCC	3273 1044
3274 1045	ATG AAG AGA TCA ATT GGG GGA AAC TTC AAG TCA AGT GAG CTA AGA TCT GAG TTC TCA AGT	3333 1064
3334 1065	CTG CAT GAA GAC TGG CTC AAG TCA TTG GTG GGT ATT TCT CGA GTC GCC CAC AAC AAT GCC	3393 1084
3394 1085	CCC ATT GGC GAA GAT GAC TAC GAC ACT TTG TTT TGC TTA TGG CAT GAG ATC AAA TCC TCT	3453 1104
3454 1105	TAC TGT GTT CCC AAA TAA ATG GCT ATG ACA TAA CACTATCTGAAGCTCGTTAAGTCTTTTGCCTCTCT	3521 5
3522 1	G ATG TTT ATT CCT CTT AAA AAA TGC TTA TAT ATC AAA AAA TTG TTT CCT CGA TTA AAA	3579 19
3580 20	AAA AAA AAA AAA AAA AAA AAA AAA AAA	3606 28

Figure 9 (Continued)

**MINIMUM OF CLIENT / 100 P. 001**

1	MAFAPKSTANTAN	MOSE	2	EMK	3	EMK	4	EMK	5	EMK	6	EMK	7	EMK	8	EMK	9	EMK	10	EMK	11	EMK	12	EMK	13	EMK	14	EMK	15	EMK	16	EMK	17	EMK	18	EMK	19	EMK	20	EMK	21	EMK	22	EMK	23	EMK	24	EMK	25	EMK	26	EMK	27	EMK	28	EMK	29	EMK	30	EMK	31	EMK	32	EMK	33	EMK	34	EMK	35	EMK	36	EMK	37	EMK	38	EMK	39	EMK	40	EMK	41	EMK	42	EMK	43	EMK	44	EMK	45	EMK	46	EMK	47	EMK	48	EMK	49	EMK	50	EMK	51	EMK	52	EMK	53	EMK	54	EMK	55	EMK	56	EMK	57	EMK	58	EMK	59	EMK	60	EMK	61	EMK	62	EMK	63	EMK	64	EMK	65	EMK	66	EMK	67	EMK	68	EMK	69	EMK	70	EMK	71	EMK	72	EMK	73	EMK	74	EMK	75	EMK	76	EMK	77	EMK	78	EMK	79	EMK	80	EMK	81	EMK	82	EMK	83	EMK	84	EMK	85	EMK	86	EMK	87	EMK	88	EMK	89	EMK	90	EMK	91	EMK	92	EMK	93	EMK	94	EMK	95	EMK	96	EMK	97	EMK	98	EMK	99	EMK	100	EMK	101	EMK	102	EMK	103	EMK	104	EMK	105	EMK	106	EMK	107	EMK	108	EMK	109	EMK	110	EMK	111	EMK	112	EMK	113	EMK	114	EMK	115	EMK	116	EMK	117	EMK	118	EMK	119	EMK	120	EMK	121	EMK	122	EMK	123	EMK	124	EMK	125	EMK	126	EMK	127	EMK	128	EMK	129	EMK	130	EMK	131	EMK	132	EMK	133	EMK	134	EMK	135	EMK	136	EMK	137	EMK	138	EMK	139	EMK	140	EMK	141	EMK	142	EMK	143	EMK	144	EMK	145	EMK	146	EMK	147	EMK	148	EMK	149	EMK	150	EMK	151	EMK	152	EMK	153	EMK	154	EMK	155	EMK	156	EMK	157	EMK	158	EMK	159	EMK	160	EMK	161	EMK	162	EMK	163	EMK	164	EMK	165	EMK	166	EMK	167	EMK	168	EMK	169	EMK	170	EMK	171	EMK	172	EMK	173	EMK	174	EMK	175	EMK	176	EMK	177	EMK	178	EMK	179	EMK	180	EMK	181	EMK	182	EMK	183	EMK	184	EMK	185	EMK	186	EMK	187	EMK	188	EMK	189	EMK	190	EMK	191	EMK	192	EMK	193	EMK	194	EMK	195	EMK	196	EMK	197	EMK	198	EMK	199	EMK	200	EMK	201	EMK	202	EMK	203	EMK	204	EMK	205	EMK	206	EMK	207	EMK	208	EMK	209	EMK	210	EMK	211	EMK	212	EMK	213	EMK	214	EMK	215	EMK	216	EMK	217	EMK	218	EMK	219	EMK	220	EMK	221	EMK	222	EMK	223	EMK	224	EMK	225	EMK	226	EMK	227	EMK	228	EMK	229	EMK	230	EMK	231	EMK	232	EMK	233	EMK	234	EMK	235	EMK	236	EMK	237	EMK	238	EMK	239	EMK	240	EMK	241	EMK	242	EMK	243	EMK	244	EMK	245	EMK	246	EMK	247	EMK	248	EMK	249	EMK	250	EMK	251	EMK	252	EMK	253	EMK	254	EMK	255	EMK	256	EMK	257	EMK	258	EMK	259	EMK	260	EMK	261	EMK	262	EMK	263	EMK	264	EMK	265	EMK	266	EMK	267	EMK	268	EMK	269	EMK	270	EMK	271	EMK	272	EMK	273	EMK	274	EMK	275	EMK	276	EMK	277	EMK	278	EMK	279	EMK</
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CTATATATAAAAGAAATGAAAGATATATATTGTTTTTTCATTTATCAAAC	100
AAAACAACAAGACTTTTTTTTACTTTTTTACATTGGTCAACAAAATACAA	150
GATAAACGACATCGTTTAATCATTTCCTCAATTTTACCCCTAAGTTTAACA	200
CCTAGAACCTTCTCCATCTTCGCAAGCACAGCCTGATTAGGAACAGCTTT	250
ACCATTCTCATATTCTGAACTACCTGAGTCCTCTCATTGATCTGTTTCG	300
CCAAATCCGCTTGTGACATCTTCTTCTCCAATCTCGCTTTCTGTATCATC	350
AACCTCACCTCTGCTTTTACACGATCCATCGCCGAGGCTCTGTTTCTTC	400
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TATAGGAGTAGATGGCGATGTTCTGCTCCAGAAACACCGGGATGCGTC	1950
CACGTGCTTCTCGCTTGAAGCGAGTTCTGGAGGATGAAATGACTTTTAAG	2000
GAGGATAAGGTTCTGTATTGGACTCTAACAAAAGGCTGAAAATGCTCCA	2050
GGATCCGGTTTGTGGAGAGAAGAAAGAAGTAACGAAGGAACCAATTTG	2100
AATGGCTTGAGTCTTCTCGAATCAGGGATGCCAATAGAAGACGTCTGAT	2150
GATCCCTTTACGATAGAAAGACCTTACACATACCACCTGATGTTTTCAA	2200

Figure 11

GAAAATGTCTGCATCACAAAAGCAATATTGGAGTGTAAAGAGTGAATATA	2250
TGGACATTGTGCTTTTCTTTAAAGTGGTTAGTAACATTAACTAGTGTT	2300
CAATCCATTTCTCAATGTGATTTGTTCACTTACATCTGTTTACGTTATG	2350
CTCTTCTCAGGGGAAATTTTATGAGCTGTATGAGCTAGATGCCGAATTAG	2400
GTCACAAGGAGCTTGACTGGAAGATGACCATGAGTGGTGTGGGAAAATGC	2450
AGACAGGTAAATTAGTTGAAACAACCTGGCCTGCTGAATTATTGTGTCTA	2500
TAAATTTTGACACCACCTTTTGTTCAGGTTGGTATCTCTGAAAGTGGGA	2550
TAGATGAGGCAGTGCAAAAGCTATTAGCTCGTGGGTAAGGGAACCATCAT	2600
ACTTTATGGAATTCGTTTACTGCTACTTCGGCTAGGATTTAAGAAATGGA	2650
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TTATTCGTTATATAATAAGTCTTTTCATCAAGGAGTAACAAACAAAACCTT	2750
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TGCTAGTTCAGTGTAGTAACAGTTACGGATAATATATGTTTACTTGTATG	2850
TGGTACCCTCATTTTGTCTCTCATGGAGGCTTTCAGCCTTGTGTTGAAA	2900
CTGGATAGTTACATATGCTTCCAACAGAACTAGCATGCAGATTCATATG	2950
CTTTCCTATTCTACTAATTATGTATTGACACACTCGTTGTTTCTTTTGAA	3000
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TTGCAGACTGTTTTTGATCATTTCCTTTTCTGTACATTACTTTCATGCTG	3150
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GCTAGTTCAGGTATTAACCTCCATCAACAGCAAGCGAGGGAACATCGGGC	3250
CTGATGCCGTCCATCTTCTTGCTATAAAAGAGGTTTGTATTACTTATT	3300
TATCTTATCATGTTTCAGTTCATCCAAGTCCTGAAAAATTACACTCTTCTT	3350
TACCAATCTTCCATCAAGCTGTGTAAAGGATTTGGAATTAGAAAAATCATT	3400
ATTTGATGCTTTGTTTTATATGCAAGAGGTTCCCTTGAAAAGATCTGTTT	3450
AAGATTCTTTGCACTTGAAAAATCAATCTTTTAAAGTGAATCCCTACT	3500
TTCTTACAATGATCATAGTCTGCAATTGCATGTCAAGTAATATCATTCCT	3550
TGTTACTGCATCCCCCTCTTTCTTAATGACCATTGTCTATGTTGTGTTTG	3600
TCTCGTGTGCTGGAGAAAATGATAGCTGATCCAAGCTGTACATTATCATG	3650
ATTAAGTAGCTGCTCAGGAATTGCCTTTGGTTACATTGCCTAATGGTTTG	3700
ATGTCAATTTTCTTCTGAATCTTTATTTTAGATCAAAATGGAGCTACAA	3750
AAGTGTTCAACTGTGTATGGATTTGCTTTTGTGACTGTGCTGCCTTGAG	3800
GTTTTGGGTTGGGTCCATCAGCGATGATGCATCATGTGCTGCTCTTGAG	3850
CGTTATTGATGCAGGTAAGCAAGTGATTCTGTATCTTATGTGTACCATG	3900
TGACTTCTGTGCATATATTTGGGTTGCAGGAACATAATTCTGAATCACCA	3950
TTTGGTATGTTTTTCCAGGTTTCTCCAAAGGAAGTGTTATATGACAGTA	4000
AAGGTAAACTGCTTGTATCGCCAGTTGTTTTGTTAAACAGAATTTAAGGT	4050
AAATGACACTGGTTAATTTAAAGTGCATACATGTTGAAATATTGCAGGGC	4100
TATCAAGAGAAGCACAAAAGGCTCTAAGGAAATATACGTTGACAGGTACC	4150
ATTTCACTAGGCAAGCTAACTGACAATTTAACCGCTCACCGAATGATAGG	4200
TCTCTTAAACATTGCTAATGTAGATGATGTTTATGTTTCAATCTAATAGG	4250
GTCTACGGCGGTACAGTTGGCTCCAGTACCACAAGTAATGGGGGATACAG	4300
ATGCTGCTGGAGTTAGAAATATAATAGAATCTAACGGATACTTTAAAGGT	4350
TCTTCTGAATCATGGAAGTGTGCTGTTGATGGTCTAAATGAATGTGATGT	4400

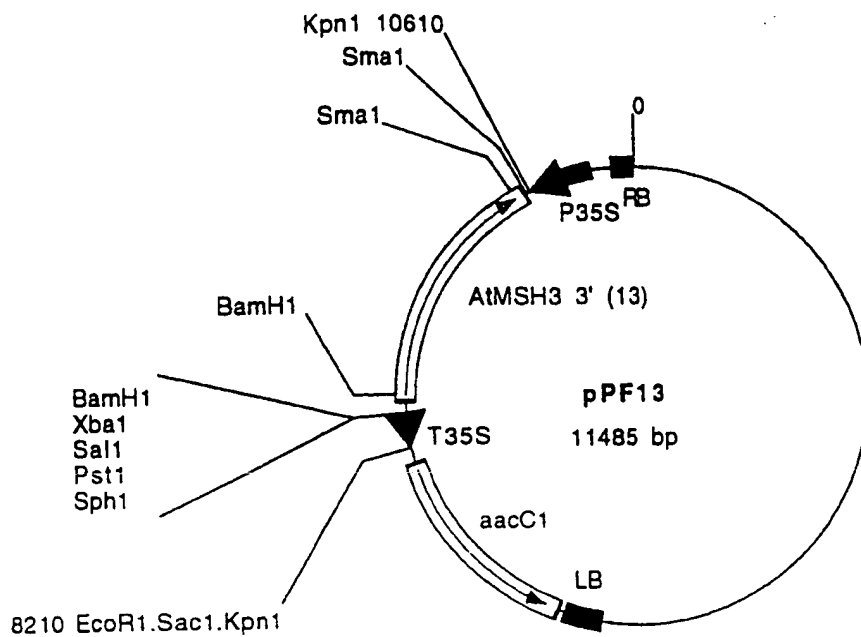
Figure 11 (Continued)

TGCCCTTAGTGCTCTTGGAGAGCTAATTAATCATCTGTCTAGGCTAAAGG	4450
TGTGTTGGCTTGTTTAGTTTTGCTTTTCACAAATTAAGCAAAGGAAGCTT	4500
TTCATAACTTACAGTTTCTATCTACTTGCAGCTAGAAGATGTACTTAAGC	4550
ATGGGGATATTTTTCCATACCAAGTTTACAGGGGTTGTCTCAGAATTGAT	4600
GGCCAGACGATGGTAAATCTTGAGATATTTAACAATAGCTGTGATGGTGG	4650
TCCTTCAGGCAAGTGCATATTTCTTTTTTGATAACTTCAACTAGAGGGCA	4700
GACATAGAAGGAAAAATCTAATACTTCGTACGGATCTCCAGTAAGTAAT	4750
AGCCGATTTTTGTTTACCTATGTAGGGACCTTGACAAATATCTTGATAA	4800
CTGTGTTAGTCCAAGTGGTAAGCGACTCTTAAGGAATTGGATCTGCCATC	4850
CACTCAAAGATGTAGAAAGCATCAATAAACGGCTTGATGTAGTTGAAGAA	4900
TTCACGGCAAAGTGCAGAAAGTATGCAAATCACTGGCCAGTATCTCCACAA	4950
ACTTCCAGACTTAGAAAGACTGCTCGGACGCATCAAGTCTAGCGTTCGAT	5000
CATCAGCCTCTGTGTTGCCTGCTCTTCTGGGAAAAAAGTGTGAAACAA	5050
CGAGTAAGTATCAATCACAAGTTTTCTGAGTAATGCCTTCCATGAGTAGT	5100
ATAGGACTAAAACATTACGGGTCTAGCTAAAGACTGTTCTCCTTCTTTTG	5150
CAATGTCTGGTTATTACATTCTCTTAACCTTATGCATTGCAGGTT	5200
AAAGCATTTGGGCAAATTGTGAAAGGGTTCAGAAGTGGAAATTGATCTGTT	5250
GTTGGCTCTACAGAAGGAATCAAATATGATGAGTTTGCTTTATAAACTCT	5300
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CTATCTTTCATACTTTACAACAAAATGTCTGTCACTACTCAAAGCAATGC	5450
ATATGGCTTAGATCTCAACTCACACCCCGAGGATCCTAAAGGGATTTGCT	5500
TTTTATTCTTAATGTTTTTGGATGGTTTGATTTATTTCTAACTTGAACCT	5550
ATTAATCTTGTACCAGAACCAAGATGTGACAGATGAAAACGCTGAAACTC	5600
TCACAATACTTATCGAACTTTTTATCGAAAGAGCAACTCAATGGTCTGAG	5650
GTCATTACACCATAAGCTGCCTAGATGTCCTGAGATCTTTTGCAATCGC	5700
AGCAAGTCTCTCTGCTGGAAGCATGGCCAGGCCTGTTATTTTCCGAAT	5750
CAGAAGCTACAGATCAGAATCAGAAAACAAAAGGCCAATACTTAAATC	5800
CAAGGACTATGGCATCCATTTGCAGTTGCAGCCGATGGTCAATTGCCGTG	5850
TCCGAATGATATACTCCTTGCGGAGGCTAGAAGAAGCAGTGGCAGCATTC	5900
ATCCTCGGTCAATTGTTACTGACGGGACCAACATGGGCGGAAAATCAACT	5950
CTTCTTCGTGCAACATGTCTGGCCGTTATCTTTGCCCAAGTTTGATACT	6000
CGTTAGATAATTACTCTATTCTTTGCAATCAGTTCTTCAACATGAATAAT	6050
AAATTCGTGTTTTCTGTCTGCAGCTTGGCTGCTACGTGCCGTGTGAGTCTT	6100
GCGAAATCTCCCTCGTGGATACTATCTTCACAAGGCTTGGCGCATCTGAT	6150
AGAATCATGACAGGAGAGAGTAAGTTTTGTTCTCAAAATACCAATTCCTC	6200
GAACTATTTACTCAGATTTTGTCTGATTGGACAAGGTGGTTTTGCTTTTT	6250
TTTAGGTACCTTTTTGGTAGAATGCACTGAGACAGCGTCAGTTCTTCAGA	6300
ATGCAACTCAGGATTCAGTAGTAATCCTTGACGAACTGGGCAGAGGAAT	6350
AGTACTTTGATGGATACGCCATTGCATACTCGGTAACCTGCTCTTCTCC	6400
TTCAACTTATACTTGTGATCAACAAAAACATGCAATTCATTTTGCTGAA	6450
ACTTATTGATTTATATCAGGTTTTTTCGTACCTGGTAGAGAAAGTTCAAT	6500
GTCGGATGCTCTTTGCAACACATTACCACCCTCTACCAAGGAATTCGCG	6550
TCTCACCCACGTGTCACCTCGAAACACATGGCTTGCGCATTCAAATCAAG	6600

Figure 11 (Continued)

ATCTGATTATCAACCACGTGGTTGTGATCAAGACCTAGTGTCTTGTACC	6650
GTTTAACCGAGGGAGCTTGTCTGAGAGCTACGGACTTCAAGTGGCACTC	6700
ATGGCTGGAATACCAAACCAAGTGGTTGAAACAGCATCAGGTGCTGCTCA	6750
AGCCATGAAGAGATCAATTGGGGAAAACCTCAAGTCAAGTGAGCTAAGAT	6800
CTGAGTTCTCAAGTCTGCATGAAGACTGGCTCAAGTCATTGGTGGGTATT	6850
TCTCGAGTCGCCCCACAACATGCCCCCATGGCGAAGATGACTACGACAC	6900
TTTGTTTTGCTTATGGCATGAGATCAAATCCTCTTACTGTGTTCCTCAAAT	6950
AAATGGCTATGACATAACACTATCTGAAGCTCGTTAAGTCTTTTGCTTCT	7000
CTGATGTTTATTCCTCTTAAAAAATGCTTATATATCAAAAAATTGTTTCC	7050
TCGATTATAACAAGATTATATATGTATCTGTGCGTTTAGCTATGGTATAT	7100
AATATATGTATGTTTCATGAGATTGGTCAAGAGAAATACTCACAAACAGTA	7150
TATTAAGAAGGAAATATGTTTATGCATTAATTTAAGTTTCAAGATAAACT	7200
GCAAATAACCTCGACTAAAGTTGCAAAGACCAAACACAAATTACAAAAC	7250
TATAAGACTTAAGTTCTGAATTCCTAAAACCAAAAAAAAAAACAGAACA	7300
TATTTTGTTGCATCTACAAACAACACAAACCTACATAGTTTATAACTTAC	7350
TCATCACTGAGATTAACATCAGAATCATTCTCCATTTCTTCATCTTCACT	7400
CTCATCATCATCACCACCACCATGATGATTCTCCTCCTCTTCACGTAACC	7450
TAGCAATCTCACTCTGAGCTCTATCAACAATCTGCTTCTTCTGCAACTCC	7500
AAATCTCTCTGAAAATCAGCTCTCATCTTCTCCAACCTCCTTCATTTGCTC	7550
TTTCTTACTCTTCTCCATCTTCTCATAAACCTTCCCAAACCTCTCAACAG	7600
AATCCGCCAACATCTTATACGAAGCAGCGTCATTAACCTTCTTCTCTCG	7650
TACTCAACCTCATCATCCTCATCCTCCTCCTCTTCAGAATCACCAGGACT	7700
ATCCATCATCTCATCAAACCCATTAGACTTATCTAAATAAACCTTAGTGT	7750
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ACTTCTCTTCAAGTCATCAATCATTCTCCTACATTGCGTCTCAGATTTC	7950
TCCATCCTTAGCTCCTCACTCACTTCTCAGCTACTTCATTCCAATCCTC	8000
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CAAGCAACACAA	8062

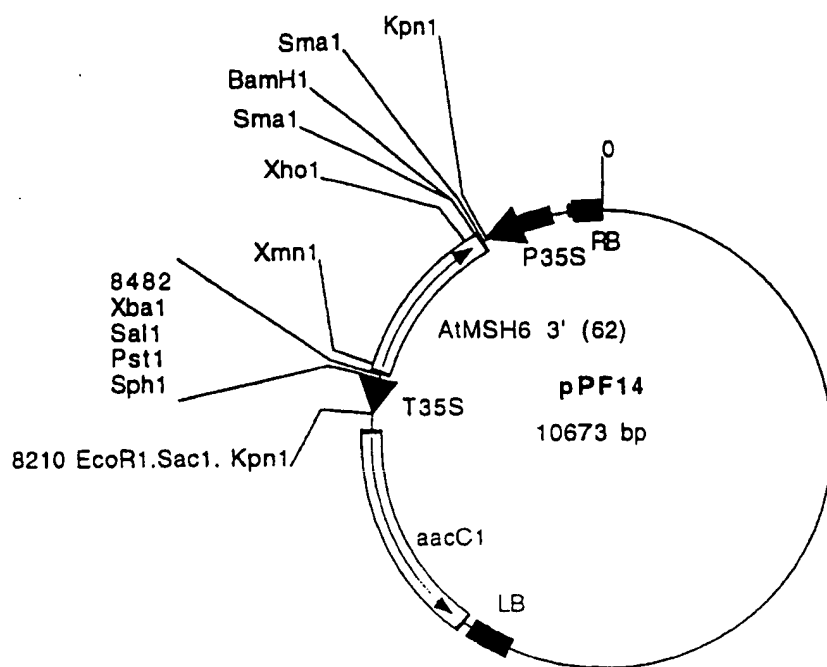
Figure 11 (Continued)



## Figure 12

**Comments/References:** AtMSH3 3' side antisense : AtMSH3 3' (13 = 2104bp) from pUC18/13 Sal1/Sst1/T4 into pCW164 BamH1/T4 in Agrobacterium LBA4404





**Figure 13**

**Comments/References:** AtMSH6 (S8) 3' side antisens : 62 Sal1/Sst1/T4 (1379bp)  
into pCW164 BamH1/T4

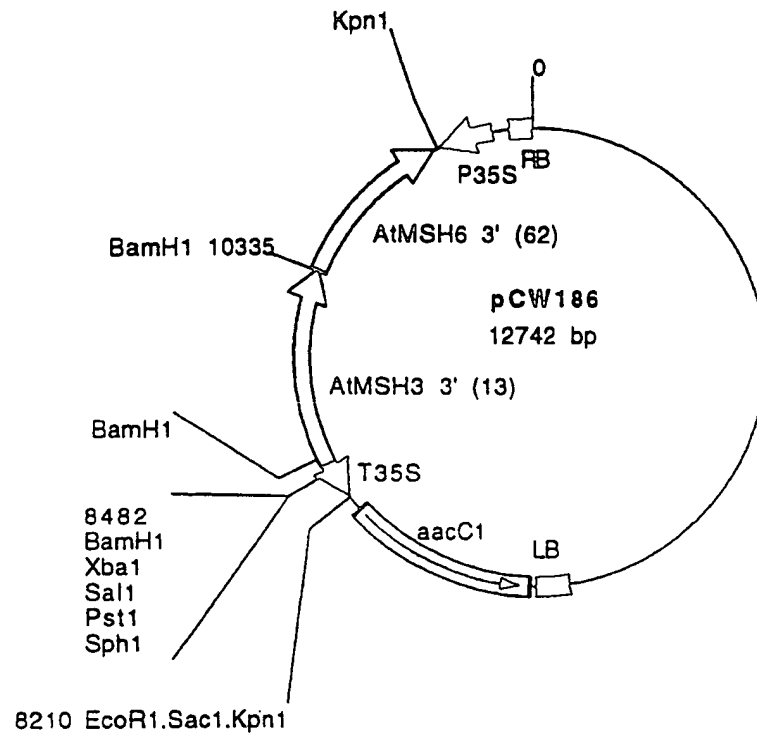


Figure 14

**Comments/References:** AtMSH6 3'/AtMSH3 3' antisense : AtMSH6 (S8) 3' side (62=1379bp) Sal1/Sst1/T4 into pPF13 (pCW164 AtMSH3 (S5) 3' side (13=2104) antisense)/Sma1. in LBA4404

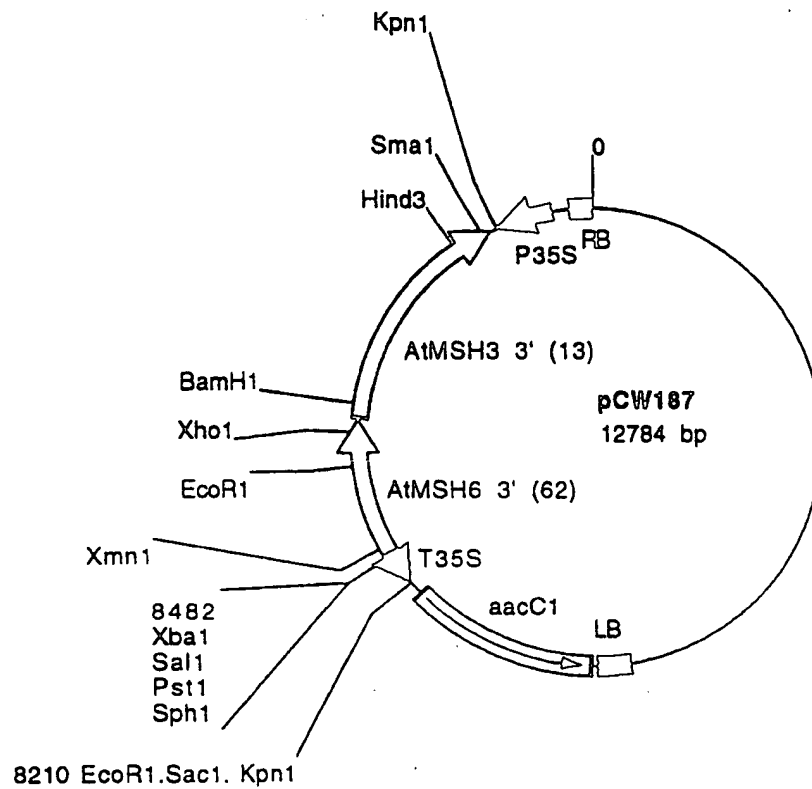
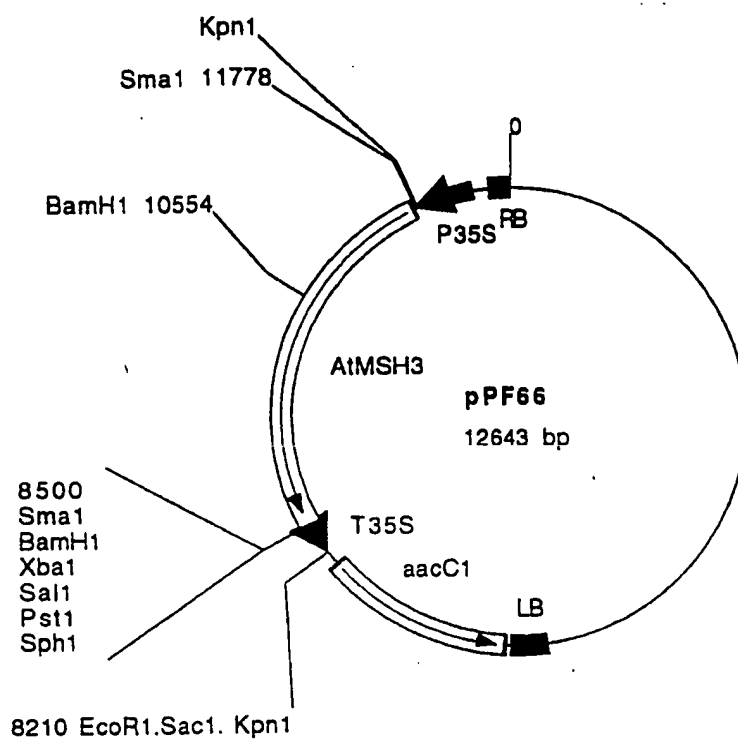
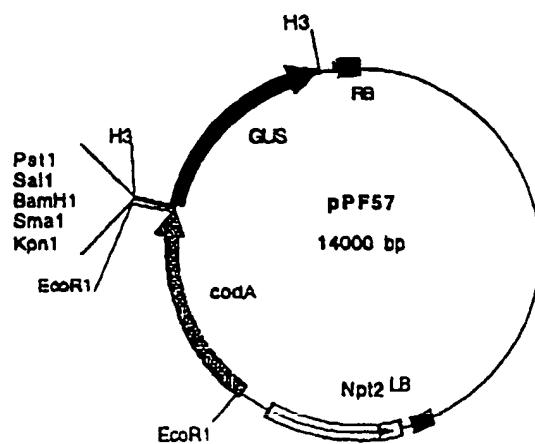


Figure 15

**Comments/References:** AtMSH3 3'/AtMSH6 3' antisens (D) : AtMSH3 (S5) 3' side (13=2104bp) Sal1/Sst1/T4 into pPF14 (AtMSH6 (S8) 3'side (62=1379bp) antisense into pCW164)/Sma1. in LBA4404

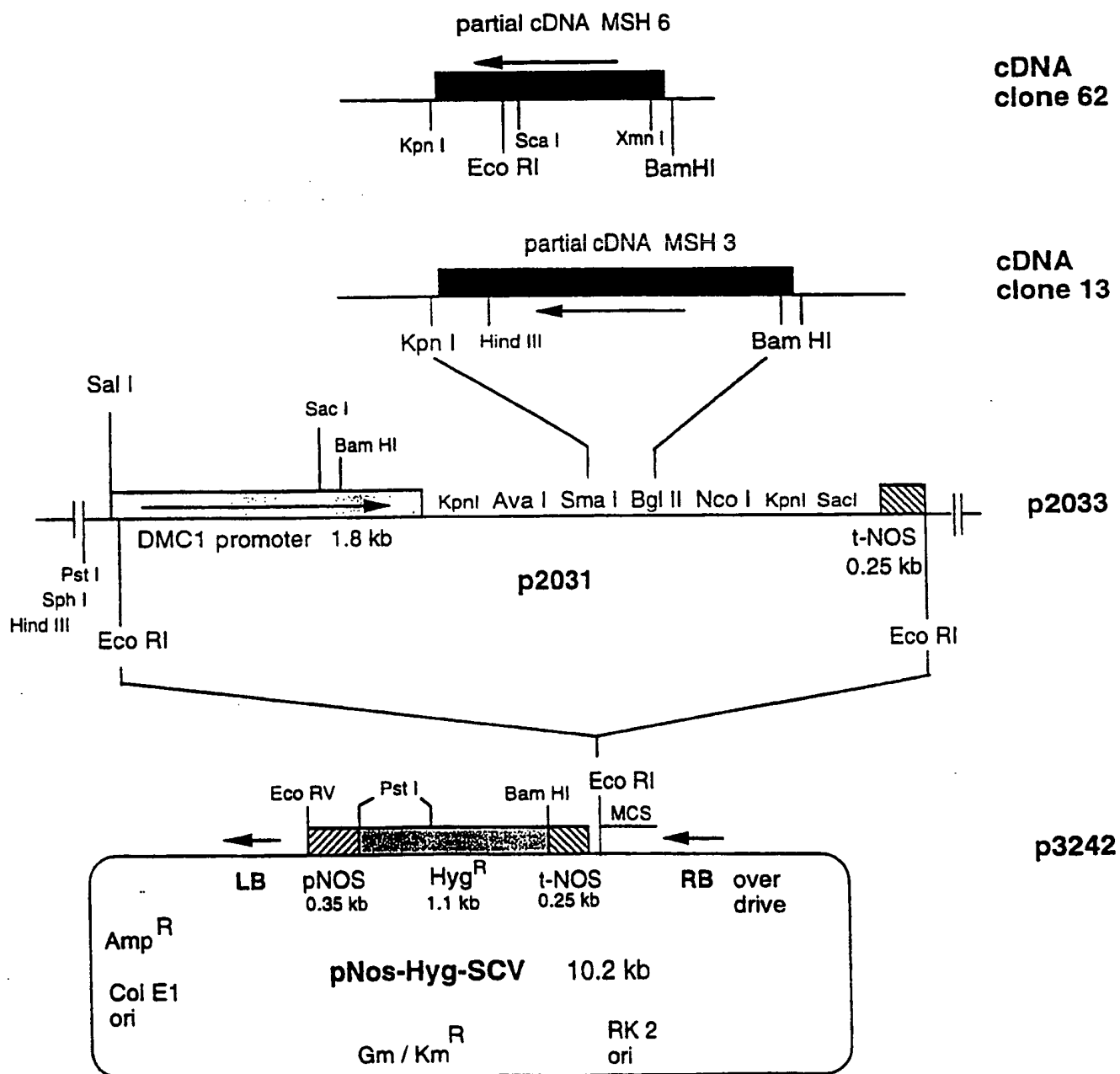
**Figure 16**

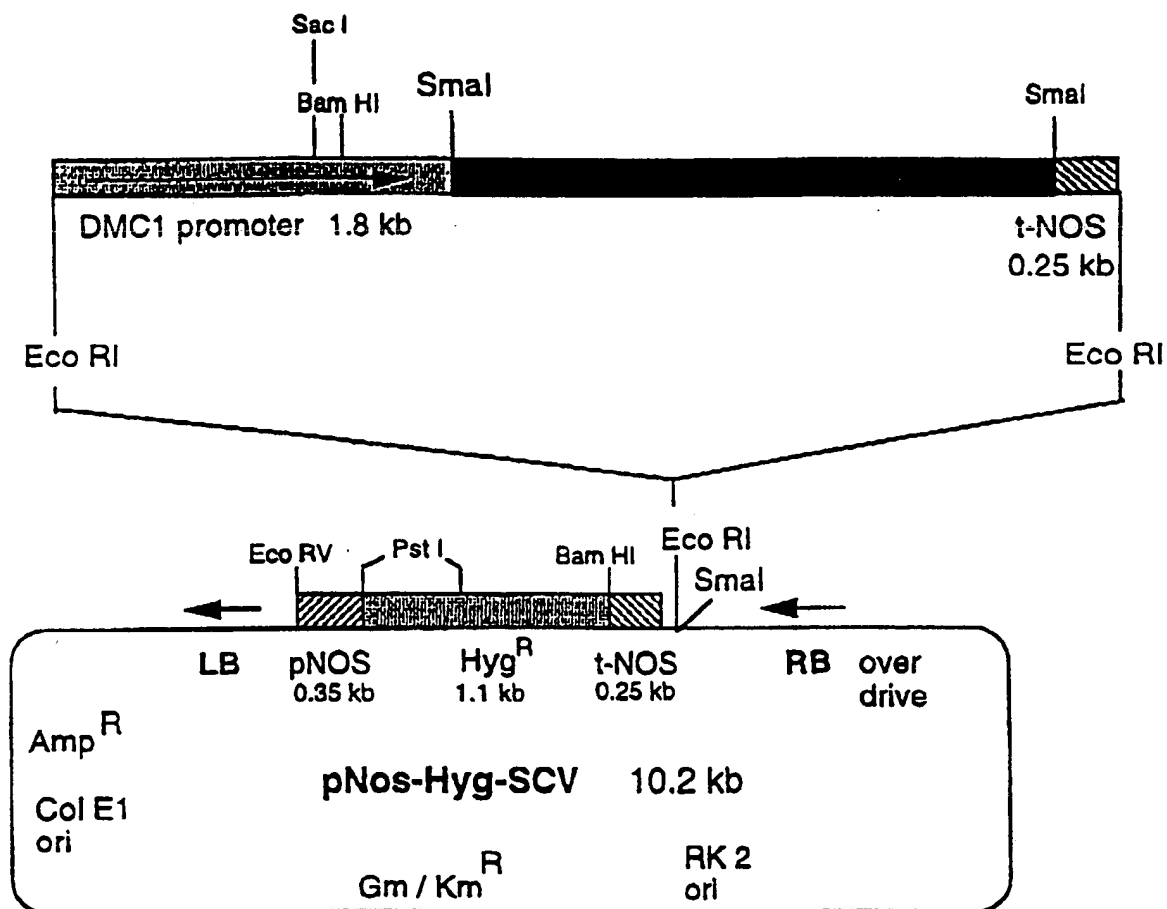
**Comments/References:** AtMSH3 (S8) complete, sense orientation : pPF26 (3342bp)  
Sma1 into pCW164 Sma1



# Figure 17

**Comments/References:** pPZP111 with codA EcoR1 cassette in EcoR1 site and Hind3 GUS cassette in Hind3 site. KanR. All genes under Promoter/terminator 35S

**Figure 18**

**Figure 19****p3243**

## SEQUENCE LISTING

<110> Rhone-Poulenc Agro; Betzner, Andreas Stefan; Doutriaux, Marie-Pascale; Freyssinet, Georges; Perez, Pascual.

<120> Methods for obtaining plant varieties

<130> 395498C

<150> PO9745

<151> 1997-10-10

<160> 98

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<302> Genetics

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<306> 963-973

<307> 1992

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<302> Genetics

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<211> 28  
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<220>  
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<220>  
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<223> Clone 52

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&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; MSH3 specific primer S52 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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27

&lt;210&gt; 15

&lt;211&gt; 2110

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana ecotype Columbia

&lt;223&gt; Clone 13

&lt;400&gt; 15

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ggttgaagaa ggttctgaga gagcaattgt atcacctgag ttttatctcg tgctctcctc 420

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cgacatacta atcacttcca gcgaccaatt tcctgagctt gctgaagctc gccaaagcagt 780

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 Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg  
 35 40 45

aag ctt ctc tcc gac cac ctc gcc gcc gcg tca ccc aaa aag cct aaa 291  
 Lys Leu Leu Ser Asp His Leu Ala Ala Ser Pro Lys Lys Pro Lys  
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 Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln  
 65 70 75 80

aga ttt ctc cag	aga ttt ctg gaa ccc	tcg ccg gag gaa tat gtt ccc	387
Arg Phe Leu Gln	Arg Phe Leu Glu Pro	Ser Pro Glu Glu Tyr Val Pro	
85	90	95	
gaa acg tca tca	tcg agg aaa tac aca	cca ttg gaa cag caa gtg gtg	435
Glu Thr Ser Ser	Ser Arg Lys Tyr Thr	Pro Leu Glu Gln Gln Val Val	
100	105	110	
gag cta aag agc aag	tac cca gat gtg gtt ttg atg	gtg gaa gtt ggt	483
Glu Leu Lys Ser	Lys Tyr Pro Asp Val	Val Leu Met Val Glu Val Gly	
115	120	125	
tac agg tac aga ttc	ttc gga gaa gac gcg	gag atc gca gca cgc gtg	531
Tyr Arg Tyr Arg	Phe Phe Gly Glu Asp	Ala Glu Ile Ala Ala Arg Val	
130	135	140	
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Leu Gly Ile Tyr	Ala His Met Asp His	Asn Phe Met Thr Ala Ser Val	
145	150	155	160
cca aca ttt cga ttg	aat ttc cat gtg aga	aga ctg gtg aat gca gga	627
Pro Thr Phe Arg	Leu Asn Phe His	Val Arg Arg Leu Val Asn Ala Gly	
165	170	175	
tac aag att ggt gta	gtg aag cag act gaa	act gca gcc att aag tcc	675
Tyr Lys Ile Gly	Val Val Lys Gln Thr	Glu Thr Ala Ala Ile Lys Ser	
180	185	190	
cat ggt gca aac cgg	acc ggc cct ttt ttc	cgg gga ctg tcg gcg ttg	723
His Gly Ala Asn	Arg Thr Gly Pro	Phe Phe Arg Gly Leu Ser Ala Leu	
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Tyr Thr Lys Ala	Thr Leu Glu Ala Ala	Glu Asp Ile Ser Gly Gly Cys	
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Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn	
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Gly Asn Ala Val Asp Glu Val Ile Ser Leu Cys Glu Lys Ile Ser Ala	
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Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn	
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Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp	
675 680 685	
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Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu	
690 695 700	

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Ile Val Ala Gly Leu Asp Glu Leu Ala Leu Ala Thr Glu His Leu Ala	
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725 730 735	
tac tac aca gat ttt aag gct gcc gtt caa gct ctt gct gca ctg gac	2355
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Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg	
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Lys Leu His Val Leu Asp Gly Val Phe Thr Arg Met Gly Ala Ser Asp	
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Ser His Ile Ile Arg Thr Cys Ser Ser Arg Ser Leu Val Ile Leu Asp	
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Glu Leu Gly Arg Gly Thr Ser Thr His Asp Gly Val Ala Ile Ala Tyr	
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13

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Ala Thr Leu Gln His Leu Leu Ala Glu Lys Arg Cys Leu Val Leu Phe  
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Val Thr His Tyr Pro Glu Ile Ala Glu Ile Ser Asn Gly Phe Pro Gly  
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Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys  
945 950 955 960

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Gly Ser Tyr Asp His Asp Asp Val Thr Tyr Leu Tyr Lys Leu Val Arg  
965 970 975

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995 1000 1005

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<213> *Arabidopsis thaliana* ecotype Columbia

<223> Polypeptide MSH3

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Pro	Lys	Ser	Pro	Thr	His	Glu	Pro	Asn	Pro	Val	Ala	Glu	Ser	Ser	Thr	20	25	30	
Pro	Pro	Pro	Lys	Ile	Ser	Ala	Thr	Val	Ser	Phe	Ser	Pro	Ser	Lys	Arg	35	40	45	
Lys	Leu	Leu	Ser	Asp	His	Leu	Ala	Ala	Ala	Ser	Pro	Lys	Lys	Pro	Lys	50	55	60	
Leu	Ser	Pro	His	Thr	Gln	Asn	Pro	Val	Pro	Asp	Pro	Asn	Leu	His	Gln	65	70	75	80
Arg	Phe	Leu	Gln	Arg	Phe	Leu	Glu	Pro	Ser	Pro	Glu	Glu	Tyr	Val	Pro	85	90	95	
Glu	Thr	Ser	Ser	Ser	Arg	Lys	Tyr	Thr	Pro	Leu	Glu	Gln	Gln	Val	Val	100	105	110	
Glu	Leu	Lys	Ser	Lys	Tyr	Pro	Asp	Val	Val	Leu	Met	Val	Glu	Val	Gly	115	120	125	
Tyr	Arg	Tyr	Arg	Phe	Phe	Gly	Glu	Asp	Ala	Glu	Ile	Ala	Ala	Arg	Val	130	135	140	
Leu	Gly	Ile	Tyr	Ala	His	Met	Asp	His	Asn	Phe	Met	Thr	Ala	Ser	Val	145	150	155	160
Pro	Thr	Phe	Arg	Leu	Asn	Phe	His	Val	Arg	Arg	Leu	Val	Asn	Ala	Gly	165	170	175	
Tyr	Lys	Ile	Gly	Val	Val	Lys	Gln	Thr	Glu	Thr	Ala	Ala	Ile	Lys	Ser	180	185	190	
His	Gly	Ala	Asn	Arg	Thr	Gly	Pro	Phe	Phe	Arg	Gly	Leu	Ser	Ala	Leu	195	200	205	
Tyr	Thr	Lys	Ala	Thr	Leu	Glu	Ala	Ala	Glu	Asp	Ile	Ser	Gly	Gly	Cys	210	215	220	
Gly	Gly	Glu	Glu	Gly	Phe	Gly	Ser	Gln	Ser	Asn	Phe	Leu	Val	Cys	Val	225	230	235	240
Val	Asp	Glu	Arg	Val	Lys	Ser	Glu	Thr	Leu	Gly	Cys	Gly	Ile	Glu	Met	245	250	255	

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 Gly Asn Leu Glu Asp Asp Lys Glu Met Lys Leu Glu Ala Ala Glu Lys  
 355 360 365  
 Gly Met Ser Cys Leu Thr Val His Thr Ile Met Asn Met Pro His Leu  
 370 375 380  
 Thr Val Gln Ala Leu Ala Leu Thr Phe Cys His Leu Lys Gln Phe Gly  
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 Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn  
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 Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg  
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 595 600 605  
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 625 630 635 640  
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 Lys Lys Leu Ala Ile Arg Asn Leu Glu Phe Leu Gln Val Ser Gly Ile  
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 Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp  
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<210> 26

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cgttccaata atggtaaaac tcaagaaaga aaccatgctt ttagtttcag tgggagagct 480

gaacttagat cagtagaaga tataggagta gatggcgatg ttcctgggtcc agaaacacca 540

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22

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1385

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&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

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34

&lt;210&gt; 29

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; MSH6 specific primer S82 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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27

&lt;210&gt; 30

&lt;211&gt; 3606

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana ecotype Columbia

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&lt;222&gt; (142)....(3468)

&lt;223&gt; AtMSH6 full-length cDNA and deduced sequence of the encoded polypeptide

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10

23

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Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys	
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380 385 390	
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Glu Ile Lys Met Glu Leu Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala	
395 400 405 410	
ttt gtt gac tgt gct gcc ttg agg ttt tgg gtt ggg tcc atc agc gat	1419
Phe Val Asp Cys Ala Ala Leu Arg Phe Trp Val Gly Ser Ile Ser Asp	
415 420 425	

25

gat gca tca tgt gct gct ctt gga gcg tta ttg atg cag gtt tct cca	1467
Asp Ala Ser Cys Ala Ala Leu Gly Ala Leu Leu Met Gln Val Ser Pro	
430 435 440	
aag gaa gtg tta tat gac agt aaa ggg cta tca aga gaa gca caa aag	1515
Lys Glu Val Leu Tyr Asp Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys	
445 450 455	
gct cta agg aaa tat acg ttg aca ggg tct acg gcg gta cag ttg gct	1563
Ala Leu Arg Lys Tyr Thr Leu Thr Gly Ser Thr Ala Val Gln Leu Ala	
460 465 470	
cca gta cca caa gta atg ggg gat aca gat gct gct gga gtt aga aat	1611
Pro Val Pro Gln Val Met Gly Asp Thr Asp Ala Ala Gly Val Arg Asn	
475 480 485 490	
ata ata gaa tct aac gga tac ttt aaa ggt tct tct gaa tca tgg aac	1659
Ile Ile Glu Ser Asn Gly Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn	
495 500 505	
tgt gct gtt gat ggt cta aat gaa tgt gat gtt gcc ctt agt gct ctt	1707
Cys Ala Val Asp Gly Leu Asn Glu Cys Asp Val Ala Leu Ser Ala Leu	
510 515 520	
gga gag cta att aat cat ctg tct agg cta aag cta gaa gat gta ctt	1755
Gly Glu Leu Ile Asn His Leu Ser Arg Leu Lys Leu Glu Asp Val Leu	
525 530 535	
aag cat ggg gat att ttt cca tac caa gtt tac agg ggt tgt ctc aga	1803
Lys His Gly Asp Ile Phe Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg	
540 545 550	
att gat ggc cag acg atg gta aat ctt gag ata ttt aac aat agc tgt	1851
Ile Asp Gly Gln Thr Met Val Asn Leu Glu Ile Phe Asn Asn Ser Cys	
555 560 565 570	
gat ggt ggt cct tca ggg acc ttg tac aaa tat ctt gat aac tgt gtt	1899
Asp Gly Gly Pro Ser Gly Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val	
575 580 585	
agt cca act ggt aag cga ctc tta agg aat tgg atc tgc cat cca ctc	1947
Ser Pro Thr Gly Lys Arg Leu Leu Arg Asn Trp Ile Cys His Pro Leu	
590 595 600	
aaa gat gta gaa agc atc aat aaa cgg ctt gat gta gtt gaa gaa ttc	1995
Lys Asp Val Glu Ser Ile Asn Lys Arg Leu Asp Val Val Glu Glu Phe	
605 610 615	
acg gca aac tca gaa agt atg caa atc act ggc cag tat ctc cac aaa	2043
Thr Ala Asn Ser Glu Ser Met Gln Ile Thr Gly Gln Tyr Leu His Lys	
620 625 630	



ctt cca gac tta gaa aga ctg ctc gga cgc atc aag tct agc gtt cga	2091
Leu Pro Asp Leu Glu Arg Leu Leu Gly Arg Ile Lys Ser Ser Val Arg	
635 640 645 650	
tca tca gcc tct gtg ttg cct gct ctt ctg ggg aaa aaa gtg ctg aaa	2139
Ser Ser Ala Ser Val Leu Pro Ala Leu Leu Gly Lys Lys Val Leu Lys	
655 660 665	
caa cga gtt aaa gca ttt ggg caa att gtg aaa ggg ttc aga agt gga	2187
Gln Arg Val Lys Ala Phe Gly Gln Ile Val Lys Gly Phe Arg Ser Gly	
670 675 680	
att gat ctg ttg ttg gct cta cag aag gaa tca aat atg atg agt ttg	2235
Ile Asp Leu Leu Leu Ala Leu Gln Lys Glu Ser Asn Met Met Ser Leu	
685 690 695	
ctt tat aaa ctc tgt aaa ctt cct ata tta gta gga aaa agc ggg cta	2283
Leu Tyr Lys Leu Cys Lys Leu Pro Ile Leu Val Gly Lys Ser Gly Leu	
700 705 710	
gag tta ttt ctt tct caa ttc gaa gca gcc ata gat agc gac ttt cca	2331
Glu Leu Phe Leu Ser Gln Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro	
715 720 725 730	
aat tat cag aac caa gat gtg aca gat gaa aac gct gaa act ctc aca	2379
Asn Tyr Gln Asn Gln Asp Val Thr Asp Glu Asn Ala Glu Thr Leu Thr	
735 740 745	
ata ctt atc gaa ctt ttt atc gaa aga gca act caa tgg tct gag gtc	2427
Ile Leu Ile Glu Leu Phe Ile Glu Arg Ala Thr Gln Trp Ser Glu Val	
750 755 760	
att cac acc ata agc tgc cta gat gtc ctg aga tct ttt gca atc gca	2475
Ile His Thr Ile Ser Cys Leu Asp Val Leu Arg Ser Phe Ala Ile Ala	
765 770 775	
gca agt ctc tct gct gga agc atg gcc agg cct gtt att ttt ccc gaa	2523
Ala Ser Leu Ser Ala Gly Ser Met Ala Arg Pro Val Ile Phe Pro Glu	
780 785 790	
tca gaa gct aca gat cag aat cag aaa aca aaa ggg cca ata ctt aaa	2571
Ser Glu Ala Thr Asp Gln Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys	
795 800 805 810	
atc caa gga cta tgg cat cca ttt gca gtt gca gcc gat ggt caa ttg	2619
Ile Gln Gly Leu Trp His Pro Phe Ala Val Ala Ala Asp Gly Gln Leu	
815 820 825	
cct gtt ccg aat gat ata ctc ctt ggc gag gct aga aga agc agt ggc	2667
Pro Val Pro Asn Asp Ile Leu Leu Gly Glu Ala Arg Arg Ser Ser Gly	
830 835 840	

agc att cat cct cgg tca ttg tta ctg acg gga cca aac atg ggc gga	2715
Ser Ile His Pro Arg Ser Leu Leu Leu Thr Gly Pro Asn Met Gly Gly	
845 850 855	
aaa tca act ctt ctt cgt gca aca tgt ctg gcc gtt atc ttt gcc caa	2763
Lys Ser Thr Leu Leu Arg Ala Thr Cys Leu Ala Val Ile Phe Ala Gln	
860 865 870	
ctt ggc tgc tac gtg ccg tgt gag tct tgc gaa atc tcc ctc gtg gat	2811
Leu Gly Cys Tyr Val Pro Cys Glu Ser Cys Glu Ile Ser Leu Val Asp	
875 880 885 890	
act atc ttc aca agg ctt ggc gca tct gat aga atc atg aca gga gag	2859
Thr Ile Phe Thr Arg Leu Gly Ala Ser Asp Arg Ile Met Thr Gly Glu	
895 900 905	
agt acc ttt ttg gta gaa tgc act gag aca gcg tca gtt ctt cag aat	2907
Ser Thr Phe Leu Val Glu Cys Thr Glu Thr Ala Ser Val Leu Gln Asn	
910 915 920	
gca act cag gat tca cta gta atc ctt gac gaa ctg ggc aga gga act	2955
Ala Thr Gln Asp Ser Leu Val Ile Leu Asp Glu Leu Gly Arg Gly Thr	
925 930 935	
agt act ttc gat gga tac gcc att gca tac tcg gtt ttt cgt cac ctg	3003
Ser Thr Phe Asp Gly Tyr Ala Ile Ala Tyr Ser Val Phe Arg His Leu	
940 945 950	
gta gag aaa gtt caa tgt cgg atg ctc ttt gca aca cat tac cac cct	3051
Val Glu Lys Val Gln Cys Arg Met Leu Phe Ala Thr His Tyr His Pro	
955 960 965 970	
ctc acc aag gaa ttc gcg tct cac cca cgt gtc acc tcg aaa cac atg	3099
Leu Thr Lys Glu Phe Ala Ser His Pro Arg Val Thr Ser Lys His Met	
975 980 985	
gct tgc gca ttc aaa tca aga tct gat tat caa cca cgt ggt tgt gat	3147
Ala Cys Ala Phe Lys Ser Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp	
990 995 1000	
caa gac cta gtg ttc ttg tac cgt tta acc gag gga gct tgt cct gag	3195
Gln Asp Leu Val Phe Leu Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu	
1005 1010 1015	
agc tac gga ctt caa gtg gca ctc atg gct gga ata cca aac caa gtg	3243
Ser Tyr Gly Leu Gln Val Ala Leu Met Ala Gly Ile Pro Asn Gln Val	
1020 1025 1030	
gtt gaa aca gca tca ggt gct gct caa gcc atg aag aga tca att ggg	3291
Val Glu Thr Ala Ser Gly Ala Ala Gln Ala Met Lys Arg Ser Ile Gly	
1035 1040 1045 1050	

28

gga aac ttc aag tca agt gag cta aga tct gag ttc tca agt ctg cat 3339  
 Glu Asn Phe Lys Ser Ser Glu Leu Arg Ser Glu Phe Ser Ser Leu His  
                   1055                  1060                  1065

gaa gac tgg ctc aag tca ttg gtg ggt att tct cga gtc gcc cac aac 3387  
 Glu Asp Trp Leu Lys Ser Leu Val Gly Ile Ser Arg Val Ala His Asn  
                   1070                  1075                  1080

aat gcc ccc att ggc gaa gat gac tac gac act ttg ttt tgc tta tgg 3435  
 Asn Ala Pro Ile Gly Glu Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp  
                   1085                  1090                  1095

cat gag atc aaa tcc tct tac tgt gtt ccc aaa taaatggcta 3478  
 His Glu Ile Lys Ser Ser Tyr Cys Val Pro Lys  
                   1100                  1105

tgacataaca ctatctgaag ctcgttaagt cttttgcttc tctgatgttt attcctctta 3538

aaaaatgctt atatatcaaa aaattgtttc ctcgattaaa aaaaaaaaaa aaaaaaaaaa 3598

aaaaaaaa 3606

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Met Gln Arg Gln Arg Ser Ile Leu Ser Phe Phe Gln Lys Pro Thr Ala  
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Ala Thr Thr Lys Gly Leu Val Ser Gly Asp Ala Ala Ser Gly Gly Gly  
                   20                  25                  30

Gly Ser Gly Gly Pro Arg Phe Asn Val Arg Glu Gly Asp Ala Lys Gly  
                   35                  40                  45

Asp Ala Ser Val Arg Phe Ala Val Ser Lys Ser Val Asp Glu Val Arg  
   50                  55                  60

Gly Thr Asp Thr Pro Pro Glu Lys Val Pro Arg Arg Val Leu Pro Ser  
   65                  70                  75                  80

Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp Ala Ser Ser Leu Phe Ser  
                   85                  90                  95

Asn Ile Met His Lys Phe Val Lys Val Asp Asp Arg Asp Cys Ser Gly  
                   100                  105                  110

Glu Arg Ser Arg Glu Asp Val Val Pro Leu Asn Asp Ser Ser Leu Cys  
                   115                  120                  125

Met Lys Ala Asn Asp Val Ile Pro Gln Phe Arg Ser Asn Asn Gly Lys  
 130 135 140

Thr Gln Glu Arg Asn His Ala Phe Ser Phe Ser Gly Arg Ala Glu Leu  
 145 150 155 160

Arg Ser Val Glu Asp Ile Gly Val Asp Gly Asp Val Pro Gly Pro Glu  
 165 170 175

Thr Pro Gly Met Arg Pro Arg Ala Ser Arg Leu Lys Arg Val Leu Glu  
 180 185 190

Asp Glu Met Thr Phe Lys Glu Asp Lys Val Pro Val Leu Asp Ser Asn  
 195 200 205

Lys Arg Leu Lys Met Leu Gln Asp Pro Val Cys Gly Glu Lys Lys Glu  
 210 215 220

Val Asn Glu Gly Thr Lys Phe Glu Trp Leu Glu Ser Ser Arg Ile Arg  
 225 230 235 240

Asp Ala Asn Arg Arg Arg Pro Asp Asp Pro Leu Tyr Asp Arg Lys Thr  
 245 250 255

Leu His Ile Pro Pro Asp Val Phe Lys Lys Met Ser Ala Ser Gln Lys  
 260 265 270

Gln Tyr Trp Ser Val Lys Ser Glu Tyr Met Asp Ile Val Leu Phe Phe  
 275 280 285

Lys Val Gly Lys Phe Tyr Glu Leu Tyr Glu Leu Asp Ala Glu Leu Gly  
 290 295 300

His Lys Glu Leu Asp Trp Lys Met Thr Met Ser Gly Val Gly Lys Cys  
 305 310 315 320

Arg Gln Val Gly Ile Ser Glu Ser Gly Ile Asp Glu Ala Val Gln Lys  
 325 330 335

Leu Leu Ala Arg Gly Tyr Lys Val Gly Arg Ile Glu Gln Leu Glu Thr  
 340 345 350

Ser Asp Gln Ala Lys Ala Arg Gly Ala Asn Thr Ile Ile Pro Arg Lys  
 355 360 365

Leu Val Gln Val Leu Thr Pro Ser Thr Ala Ser Glu Gly Asn Ile Gly  
 370 375 380

Pro Asp Ala Val His Leu Leu Ala Ile Lys Glu Ile Lys Met Glu Leu  
 385 390 395 400

Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala Phe Val Asp Cys Ala Ala  
 405 410 415

Leu Arg Phe Trp Val Gly Ser Ile Ser Asp Asp Ala Ser Cys Ala Ala  
 420 425 430  
 Leu Gly Ala Leu Leu Met Gln Val Ser Pro Lys Glu Val Leu Tyr Asp  
 435 440 445  
 Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys Ala Leu Arg Lys Tyr Thr  
 450 455 460  
 Leu Thr Gly Ser Thr Ala Val Gln Leu Ala Pro Val Pro Gln Val Met  
 465 470 475 480  
 Gly Asp Thr Asp Ala Ala Gly Val Arg Asn Ile Ile Glu Ser Asn Gly  
 485 490 495  
 Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn Cys Ala Val Asp Gly Leu  
 500 505 510  
 Asn Glu Cys Asp Val Ala Leu Ser Ala Leu Gly Glu Leu Ile Asn His  
 515 520 525  
 Leu Ser Arg Leu Lys Leu Glu Asp Val Leu Lys His Gly Asp Ile Phe  
 530 535 540  
 Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg Ile Asp Gly Gln Thr Met  
 545 550 555 560  
 Val Asn Leu Glu Ile Phe Asn Asn Ser Cys Asp Gly Gly Pro Ser Gly  
 565 570 575  
 Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val Ser Pro Thr Gly Lys Arg  
 580 585 590  
 Leu Leu Arg Asn Trp Ile Cys His Pro Leu Lys Asp Val Glu Ser Ile  
 595 600 605  
 Asn Lys Arg Leu Asp Val Val Glu Glu Phe Thr Ala Asn Ser Glu Ser  
 610 615 620  
 Met Gln Ile Thr Gly Gln Tyr Leu His Lys Leu Pro Asp Leu Glu Arg  
 625 630 635 640  
 Leu Leu Gly Arg Ile Lys Ser Ser Val Arg Ser Ser Ala Ser Val Leu  
 645 650 655  
 Pro Ala Leu Leu Gly Lys Lys Val Leu Lys Gln Arg Val Lys Ala Phe  
 660 665 670  
 Gly Gln Ile Val Lys Gly Phe Arg Ser Gly Ile Asp Leu Leu Leu Ala  
 675 680 685  
 Leu Gln Lys Glu Ser Asn Met Met Ser Leu Leu Tyr Lys Leu Cys Lys  
 690 695 700

Leu Pro Ile Leu Val Gly Lys Ser Gly Leu Glu Leu Phe Leu Ser Gln  
 705 710 715 720  
 Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro Asn Tyr Gln Asn Gln Asp  
 725 730 735  
 Val Thr Asp Glu Asn Ala Glu Thr Leu Thr Ile Leu Ile Glu Leu Phe  
 740 745 750  
 Ile Glu Arg Ala Thr Gln Trp Ser Glu Val Ile His Thr Ile Ser Cys  
 755 760 765  
 Leu Asp Val Leu Arg Ser Phe Ala Ile Ala Ala Ser Leu Ser Ala Gly  
 770 775 780  
 Ser Met Ala Arg Pro Val Ile Phe Pro Glu Ser Glu Ala Thr Asp Gln  
 785 790 795 800  
 Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys Ile Gln Gly Leu Trp His  
 805 810 815  
 Pro Phe Ala Val Ala Ala Asp Gly Gln Leu Pro Val Pro Asn Asp Ile  
 820 825 830  
 Leu Leu Gly Glu Ala Arg Arg Ser Ser Gly Ser Ile His Pro Arg Ser  
 835 840 845  
 Leu Leu Leu Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Leu Leu Arg  
 850 855 860  
 Ala Thr Cys Leu Ala Val Ile Phe Ala Gln Leu Gly Cys Tyr Val Pro  
 865 870 875 880  
 Cys Glu Ser Cys Glu Ile Ser Leu Val Asp Thr Ile Phe Thr Arg Leu  
 885 890 895  
 Gly Ala Ser Asp Arg Ile Met Thr Gly Glu Ser Thr Phe Leu Val Glu  
 900 905 910  
 Cys Thr Glu Thr Ala Ser Val Leu Gln Asn Ala Thr Gln Asp Ser Leu  
 915 920 925  
 Val Ile Leu Asp Glu Leu Gly Arg Gly Thr Ser Thr Phe Asp Gly Tyr  
 930 935 940  
 Ala Ile Ala Tyr Ser Val Phe Arg His Leu Val Glu Lys Val Gln Cys  
 945 950 955 960  
 Arg Met Leu Phe Ala Thr His Tyr His Pro Leu Thr Lys Glu Phe Ala  
 965 970 975  
 Ser His Pro Arg Val Thr Ser Lys His Met Ala Cys Ala Phe Lys Ser  
 980 985 990

32

Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp Gln Asp Leu Val Phe Leu  
 995 1000 1005

Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu Ser Tyr Gly Leu Gln Val  
 1010 1015 1020

Ala Leu Met Ala Gly Ile Pro Asn Gln Val Val Glu Thr Ala Ser Gly  
 1025 1030 1035 1040

Ala Ala Gln Ala Met Lys Arg Ser Ile Gly Glu Asn Phe Lys Ser Ser  
 1045 1050 1055

Glu Leu Arg Ser Glu Phe Ser Ser Leu His Glu Asp Trp Leu Lys Ser  
 1060 1065 1070

Leu Val Gly Ile Ser Arg Val Ala His Asn Asn Ala Pro Ile Gly Glu  
 1075 1080 1085

Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp His Glu Ile Lys Ser Ser  
 1090 1095 1100

Tyr Cys Val Pro Lys  
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<210> 32  
 <211> 24  
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<220>  
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 microsatellite

<400> 32

accatgcata gcttaaactt cttg

24

<210> 33  
 <211> 22  
 <212> DNA  
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<220>  
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 microsatellite

<400> 33

acataaccac aaataggggt gc

22

<210> 34  
<211> 18  
<212> DNA  
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<220>  
<223> Forward primer DMCIN-A for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

<400> 34

gaagcgatat tggttcgtg 18

<210> 35  
<211> 18  
<212> DNA  
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<220>  
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<400> 35

agattgcgag aacattcc 18

<210> 36  
<211> 31  
<212> DNA  
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<220>  
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<400> 36

acgcgctcgac tcagctatga gattactcgt g 31

<210> 37  
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<212> DNA  
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<220>  
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<400> 37

gctctagatt tctcgtctta agactctct 29



<210> 38  
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<220>  
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<400> 38

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<210> 39  
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<220>  
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<400> 39

tcccccgggc tcgagagatc tccatgggtt cttcagctct atgaatcc 48

<210> 40  
<211> 26  
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<220>  
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<400> 40

acgcgtcgac gaattcgcaa gtgggg 26

<210> 41  
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<220>  
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<400> 41

tccatggaga tctccccgggt accgatttgc ttcgaggg

38

<210> 42

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of ATEAT1 SSLP marker in Arabidopsis thaliana subspecies

<400> 42

gccactgcgt gaatgatatg

20

<210> 43

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of ATEAT1 SSLP marker in Arabidopsis thaliana subspecies

<400> 43

cgaacagcca acattaattc cc

22

<210> 44

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA63 SSLP marker in Arabidopsis thaliana subspecies

<400> 44

aaccaaggca cagaagcg

18

<210> 45

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA63 SSLP marker in Arabidopsis thaliana subspecies

<400> 45

acccaagtga tcgccacc

18

<210> 46

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA248 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 46

taccgaacca aaacacaaag g

21

<210> 47

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA248 SSLP marker in  
Arabidopsis thaliana subspecies

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tctgtatctc ggtgaattct cc

22

<210> 48

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA128 SSLP marker in  
Arabidopsis thaliana subspecies

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ggtctgttga tgtcgtaagt cg

22

<210> 49

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA128 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 49

atcttgaaac ctttagggag gg 22

<210> 50  
<211> 22  
<212> DNA  
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<220>  
<223> Forward primer for PCR amplification of NGA280 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 50

ctgatctcac ggacaatagt gc 22

<210> 51  
<211> 20  
<212> DNA  
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<220>  
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Arabidopsis thaliana subspecies

<400> 51

ggctccataa aaagtgcacc 20

<210> 52  
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<212> DNA  
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<220>  
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Arabidopsis thaliana subspecies

<400> 52

ctccagttgg aagctaaagg g 21

<210> 53  
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<212> DNA  
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<220>  
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Arabidopsis thaliana subspecies

<400> 53

tgtttttttag gacaaatggc g 21

<210> 54  
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<212> DNA  
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<220>  
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Arabidopsis thaliana subspecies

<400> 54

ccttcacatc caaaacccac 20

<210> 55  
<211> 20  
<212> DNA  
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<220>  
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Arabidopsis thaliana subspecies

<400> 55

gcacataccc acaaccagaa 20

<210> 56  
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<220>  
<223> Forward primer for PCR amplification of NGA1126 SSLP marker  
in Arabidopsis thaliana subspecies

<400> 56

cgctacgctt ttcggtaaag 20

<210> 57  
<211> 20  
<212> DNA  
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<220>  
<223> Reverse primer for PCR amplification of NGA1126 SSLP marker  
in *Arabidopsis thaliana* subspecies

<400> 57

gcacagtcca agtcacaacc 20

<210> 58  
<211> 20  
<212> DNA  
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<220>  
<223> Forward primer for PCR amplification of NGA361 SSLP marker in  
*Arabidopsis thaliana* subspecies

<400> 58

aaagagatga gaatttggac 20

<210> 59  
<211> 23  
<212> DNA  
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<220>  
<223> Reverse primer for PCR amplification of NGA361 SSLP marker in  
*Arabidopsis thaliana* subspecies

<400> 59

acatatcaat atattaaagt agc 23

<210> 60  
<211> 18  
<212> DNA  
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<220>  
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*Arabidopsis thaliana* subspecies

<400> 60

tcgtctactg cactgccg

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Arabidopsis thaliana subspecies

<400> 61

gaggacatgt ataggagcct cg

22

<210> 62  
<211> 20  
<212> DNA  
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<220>  
<223> Forward primer for PCR amplification of AthBIO2 SSLP marker  
in Arabidopsis thaliana subspecies

<400> 62

tgacctcctc ttccatggag

20

<210> 63  
<211> 22  
<212> DNA  
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<220>  
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in Arabidopsis thaliana subspecies

<400> 63

ttaacagaaa cccaaagctt tc

22

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<220>  
<223> Forward primer for PCR amplification of AthUBIQUE SSLP marker  
in Arabidopsis thaliana subspecies

<400> 64

aggcaaatgt ccatttcatt g

21

<210> 65

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<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of AthUBIQUE SSLP marker in Arabidopsis thaliana subspecies

<400> 65

acgacatggc agatttctcc

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<223> Reverse primer for PCR amplification of NGA172 SSLP marker in Arabidopsis thaliana subspecies

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<211> 21

<212> DNA

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<223> Forward primer for PCR amplification of NGA126 SSLP marker in  
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<223> Reverse primer for PCR amplification of NGA126 SSLP marker in  
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<210> 70

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<212> DNA

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<223> Forward primer for PCR amplification of NGA162 SSLP marker in  
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in Arabidopsis thaliana subspecies

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&lt;220&gt;

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&lt;210&gt; 83

&lt;211&gt; 22

&lt;212&gt; DNA

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<223> Forward primer for PCR amplification of NGA106 SSLP marker in  
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<b>(21) International Application Number:</b> PCT/EP98/06977 <b>(22) International Filing Date:</b> 9 October 1998 (09.10.98)  <b>(30) Priority Data:</b> PO 9745 10 October 1997 (10.10.97) AU  <b>(71) Applicant (for all designated States except US):</b> RHONE-POULENC AGRO [FR/FR]; 14/20, rue Pierre Baizet, F-69009 Lyon (FR).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> DOUTRIAUX, Marie-Pascale [FR/FR]; 64, route de Villebon, F-91160 Saulx les Chartreux (FR). BETZNER, Andreas, Stefan [AU/AU]; 40 Dallachy Place, Page, ACT 2614 (AU). FREYSSINET, Georges [FR/FR]; 21, rue de Nervieux, F-69450 Saint Cyr au Mont d'Or (FR). PEREZ, Pascal [FR/FR]; 17, chemin de la Pradelle, Varennes, F-63450 Chanonat (FR).  <b>(74) Agent:</b> GENIN, Patrick; Rhône-Poulenc Agro, DPI, 14/20, rue Pierre Baizet, F-69009 Lyon (FR).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 24 June 1999 (24.06.99)	
<b>(54) Title: METHODS FOR OBTAINING PLANT VARIETIES</b>		
<b>(57) Abstract</b>		
An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.		

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/06977

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/29 C07K14/415 C12N15/10 C12N5/04  
C12N5/14 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 15381 A (CHAPELLE ALBERT DE ;UNIV JOHNS HOPKINS (US)) 8 June 1995 see the whole document ---	1-5, 33-35
A	WO 97 37011 A (SETRATECH S A R L ;BORTS RHONA HARRIET (GB); LOUIS EDWARD JOHN (GB) 9 October 1997 see abstract see the whole document ---	1-35
A	WO 90 07576 A (SETRATECH) 12 July 1990  see the whole document and specially page 5, line 16-31, examples 3-4 --- -/--	1,7-27, 31,32

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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## INTERNATIONAL SEARCH REPORT

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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	PROLLA T A ET AL: "MLH1, PMS1, AND MSH2 INTERACTIONS DURING THE INITIATION OF DNA MISMATCH REPAIR IN YEAST" SCIENCE, vol. 265, 19 August 1994, pages 1091-1093, XP000676403 cited in the application	1,2,4,5
A	A. WATANABE ET AL., : "Genomic organization and expression of the human MSH3 gene" GENOMICS, vol. 31, 1996, pages 311-318, XP002099967 see the whole document and specially Fig.3	1-6
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